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STUDIES IN PERONOSPORACEÆ

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COLOCASIÆ BLIGHT CAUSED BY PHYTOPHTHORA COLOCASIÆ RAC.

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I. Description of the Disease.

Phytophthora Colocasiæ Rac. was first described by Raciborski[1] in 1900. In Java, according to this author, it is widely distributed as a parasite on the leaves of *Colocasia esculenta* (= *C. antiquorum* Schott.) but does not cause much damage to the plant. His description is brief and practically confined to the naked eye appearance of the infected leaf and the sporangial stage of the fungus. It was not, apparently, obtained elsewhere than on the leaves of the host and was not cultivated.

The parasite does not appear to have been reported outside Java until 1907, when it was recorded from India, in collections made in 1905[2]. More recently Sawada has encountered it in Formosa[3]; his description is in Japanese but illustrated by two plates, these being, so far as we know, the only published figures of the fungus. Sawada, on what appear to us to be entirely insufficient

[1] Raciborski, M. Parasitische Algen und Pilze Javas, I, 1900, p. 9.

[2] Sydow, H. & P., and Butler, E. J. Fungi Indiæ Orientalis, II. Ann. Mycol. V, 1907, p. 512.

[3] Sawada, K. Infection of Taro. Rep. from Special Reports of the Formosan Agric. Exper. Station, II, 1911 (Japanese). We are indebted to Mr. S. Kamisaka of Bombay for a translation of this paper.

grounds, transferred the species to the genus *Kawakamia*, a genus founded by K. Miyabe for a fungus parasitic on the stems of *Cyperus tegetiformis*[1], and itself doubtfully tenable.

In India the distribution is wide, from Travancore on the south to Dehra Dun and Rangoon on the north and east, and in Bengal it is one of the commonest of the *Peronosporaceæ*. It is found everywhere in the plots of *kachu* (*Colocasia antiquorum* Schott.) seen towards the middle of the rains in most villages. It also occurs in the wild specimens of the same plant common in moist localities. Raciborski's statement that it causes little damage is too sweeping. Sometimes, in favourable weather for its development, the leaves are so rotted that the entire plant is killed. In 1909, during a period of almost continuous cloud and rain in August, a number of plants died from the disease at Pusa and the growth of the rest was brought to a standstill. Further, the parasite commonly reaches the corm and sets up a dry rot during storage. This stage was not known to Raciborski but is probably more destructive, as a rule, than the leaf attack.

The disease first manifests itself ordinarily on the leaf. Small dark roundish specks appear which widen rather rapidly by centrifugal growth. The growth may be equal all round, in which case the spot remains circular, or may be more rapid in one direction than another, when we find oval, elongated or irregular patches (Pl. I). The main veins of the leaf often check extension for a short time but are soon crossed. Two or more spots may unite and ultimately a very large part of the leaf surface may be involved. In the early stages drops of a clear yellow liquid ooze out from the surface of the spots ; later, the central portion assumes a yellowish-brown colour, dries up and may become perforated. The margins are often beautifully zoned, in different shades of brown, green and yellow ; towards the periphery these zones reveal on careful examination a delicate white haze which is caused by the sporangial stage of the fungus. This external development of the parasite is,

[1] Miyabe, K., in Kawakami, T. "Kawakamia, Miyabe, a new genus belonging to *Peronosporaceæ*." Tokyo, Shōkwabo & Co., 1904.

however, much less prominent than in, for instance, the allied potato blight, and is sometimes difficult to detect by the naked eye. As the spot widens sporangia continue to be produced at progressively increasing distances from the centre.

The above may, in mild cases, constitute the whole extent of the attack. Frequently, however, the petiole becomes infected. The course of the disease here is similar to that on the leaf-blade except that the spots soon become elongated in the direction of the axis of the stalk. The damage caused is, however, greater, for if the spot is of any considerable size, it leads to such shrinking and disorganisation of the tissues that collapse of the structure above is brought about. The leaf hangs on the stalk and rapidly decomposes. Sometimes the petiole breaks at the point of worst attack, the part below drying up so rapidly as to prevent further progress. If this does not happen extension down the petiole may continue as far as its sheathing base ; infection of the stem at this point was naturally looked for and though no definite case was seen, the ready extension down the petiole renders it not improbable. In *Phytophthora infestans* it was at one time believed that infection of the potato tubers, which is well known to occur, results from extension of the mycelium from the leaves and stalks down within the tissues. It is now more generally held to be due to direct infection from sporangia and zoospores fallen from the above-ground parts and germinating in contact with the tubers. Similarly, in *Colocasia* direct infection of the corm must occur and the more readily that it is not uncommon to find corms partly exposed at the surface of the soil. Though internal infection through the stem may occur, the fact that no actual case could be found suggests that external infection, from reproductive bodies formed on the leaves and petioles, is the more usual course.

In a few cases infection of the inflorescence was observed.

In a severe attack all the leaves may be lost and the plant killed. In milder cases the young leaves may be free from disease or only slightly involved, while the older ones are covered with patches of varying extent or are hanging rotted on their stalks.

The corms may be entirely lacking or, if formed, may be small and shrunken. Where only isolated spots occur there is little apparent injury to the plant and the corm matures normally. This is the condition which has been most frequently found. Nothing is clearer than the close connection between the intensity of the disease and the atmospheric conditions prevalent during the growth period of the host. Much the worst attack noticed at Pusa during the last six years occurred in 1909, a year when the rainfall was nearly double the normal, there was almost continuous cloud or rain during August and September, the total precipitation for these two months was 28·44 inches and the 8 A.M. relative humidity exceeded 80 per cent. on fifty-three out of sixty-one days. In the present year (1912) the disease had not appeared up to the end of September, the rainfall during the previous two months having been only 12·96 inches and the 8 A.M. relative humidity exceeding 80 per cent. on forty days only. Sawada notes that the disease is worse in shady than in sunny localities in Formosa.

Infection of the corm was first noticed in 1909. It has been observed each year since, though not to the same degree. The corm rot is sometimes apparent even in the field but more usually develops subsequently in storage. As in the tuber rot caused by *Phytophthora infestans* it appears to be, when not complicated by secondary organisms, a dry rot, the tissues being dry but rather soft to the touch. Wet rot may develop in stored corms in which *Phytophthora Colocasiæ* can be detected but this is probably due to secondary invasion by various soil organisms.

II. Microscopic characters of the disease and of its cause.

The mycelium of the parasite is present in all the parts which show external discoloration. It has been found in the leaf (including the petiole), the inflorescence and the corm. The hyphæ are large, usually 5 or 6μ, but varying from 4 to 9μ in diameter, unseptate and branch copiously. In the leaf-mesophyll they are purely intercellular but epidermal cells are often traversed by hyphæ both entering and emerging from the leaf. Haustoria

(Pl. II, Fig. 2) are sent freely into the cells of the pallisade and spongy parenchyma and sometimes into those of the epidermis. They are simple, or occasionally branched, finger-shaped processes, not differing from those of *Sclerospora* or *Pythium palmivorum* in any essential. In the petiole the hyphæ are still inter-cellular, being found especially in the air-spaces between the cells, and the haustoria are numerous and very distinct. Neither in the leaf nor in the petiole are the bundles ever penetrated. In the corm, on the other hand, not alone are hyphæ found in the storage cells, the mycelium being rather intra- than inter-cellular, but the bundles are commonly penetrated (Pl. II, Fig. 5). Haustoria-like branches extend from the inter-cellular hyphæ into the storage cells but they are often not clearly distinguishable from the ordinary branches of the mycelium, which themselves may extend from cell to cell without appearing to experience the slightest difficulty in penetrating the cell walls. This difference in habit according to the tissue attacked is of considerable interest and appears to suggest that it is not so much the structure of the wall, as the nature of the substances occurring within the cells, that determines whether penetration will take place or not.

In assimilating cells the chloroplasts commence to lose colour soon after infection takes place. Their sharpness of outline then gradually disappears and the individual plastids run together into a grumous mass in which, for a while, the starch granules are still visible. Then the cells turn brown, the phloem elements sharing in this change although not penetrated by the parasite. In the corm, as soon as rotting is well established, and in the centre of the leaf spots, starch is entirely absent from infected cells.

No trace of reproductive organs has been detected in any part of the internal mycelium. Sporangia are produced externally on short stalks, arising from the epidermis at the margins of the spots on both surfaces of the leaf blade and on the petiole. They come out in successive crops as the centrifugal spread of the fungus continues. They have not been observed occurring naturally on the surface of rotting corms but have been obtained by removing with

a sterile knife a slab from the margin of a rotting patch and dropping it into pure water in a sterile flask. In some cases a copious external growth was obtained by this treatment and mature sporangia were formed on the third day. The fungus maintains its vitality for a considerable time in the corm. Pl. III, Fig. 14 shows a hypha obtained by incubating a corm about a month after it was harvested and some three months after the disease had ceased to be active. It is probable too that corms kept under conditions which permit of moisture to accumulate, develop aerial hyphæ in store and infect their neighbours. There is no reason to doubt that the parasite can persist from one season to another in the corms and, as in the allied potato blight, infection of the new crop possibly comes in part from these diseased tubers. Resting spores, whether sexually produced oospores or the thick walled reproductive bodies known as chlamydospores, have not been found in nature, though they have been obtained in artificial cultures.

On the leaf the sporangia are borne on short stalks as shown in Pl. II, Fig. 1. They are terminal and single but sometimes the stalk continues to grow by a branch arising below the sporangium, the latter being pushed to one side and a new sporangium being formed at the tip of the branch (Pl. III, Fig. 15). The sporangiophore narrows towards the apex to an extremely slender thread, sometimes not exceeding 1·5μ in diameter, and the sporangium falls with a part of this narrow stalk attached to its base. The fructifying hyphæ emerge, as shown in the figure, not only through stomata (the usual mode of emergence in *Peronosporaceæ*) but also between or through the epidermal cells. They rarely exceed 50μ in length and, excepting in the rather infrequent case of more than one sporangium being borne, they are unbranched.

The sporangia are colourless, elliptical or pear-shaped, sometimes curved and less regular in shape than is the case with most species of *Phytophthora*. They are often very large, measuring when formed on the leaf from 38 to 60 by 18 to 26μ in diameter but varying within wider limits in culture. Sawada has observed sporangia up to 114μ in length on the leaf, but in India they rarely

exceed 60μ. The wall is thin and smooth and at the free end there is a broad blunt papilla. When mature each sporangium has a single large vacuole lying near the centre, the rest of the cell being filled with dense granular protoplasm (Pl. IV, Figs. 1 and 2). Germination may occur within half an hour after sowing in water, up to 20 large zoospores being liberated. The zoospores are more or less bean-shaped, one of the longer sides being convex and the other concave or plane (Pl. IV, Fig. 3). Each contains a small pulsating vacuole and two cilia arise near together from the concave or plane side, one projecting in front and the other behind while swimming. After swimming for some time they come to rest, round off, lose the cilia and become surrounded by a cellulose wall. At this period they measure from 10 to 13μ in diameter (Raciborski gives the measurement of the swimming spores as 15 to 18 by 9 to 12μ; Sawada 14 to 18 by 9 to 13μ). Germination then occurs by a germ-tube, which may be protruded within half an hour after the zoospore comes to rest or within an hour after the liberation of the zoospore from the sporangium. The germ-tube grows and branches for a time (Pl. III, Fig. 10) but in water dies without forming any definite mycelium. Raciborski[1] describes a peculiar type of germination in this fungus. The zoospore, after coming to rest, puts out a short tube which swells at the tip to form a sort of secondary sporangium which may continue growth by a hypha or may open to liberate one or two secondary zoospores. This recalls the germination of the zoospores of *Pythium diacarpum*[2] and is to be considered as the physiological equivalent of the process of diplanetism in the *Saprolegniaceæ*. Diplanetism is probably, as suggested by Hartog[3], an adaptation to secure wider dissemination than could be obtained by the swimming spore alone. During the period of rest between the two stages of activity the spore can be passively carried to a distance by currents of water and can then reach a suitable substratum for further development

[1] *loc. cit*, p. 10.

[2] Butler, E. J. An account of the genus *Pythium* and some *Chytridiaceæ*. Mem. Dept. of Agric. in India, Bot. Ser., I, No. 5, 1907, p. 81.

[3] Hartog, M. On the Formation and Liberation of the Zoospores in the *Saprolegniæ*, Quart. Journ. of Microscop. Science, N. S., XXVII, 1887, p. 437.

during the second active stage. In some genera of *Chytridiaceæ* the same purpose is apparently served by a period of quiescence of the spore, during which the cilia are retracted to be again protruded after a time sufficient to allow of a certain amount of passive dissemination[1]. Sawada has also observed the formation of a secondary spore germinating by a hypha on the germ-tube of the primary spore, but the formation of secondary zoospores has not been observed either by him or by us and is certainly of infrequent occurrence.

Sometimes the zoospores fail to escape, one or more being left in the sporangium. After a time these cease to move, secrete a wall and germinate in the usual manner. The germ-tube appears to be unable to pierce the sporangial wall and can only emerge through the opening (Pl. III, Fig. 11).

Recently matured sporangia germinate for the most part by giving zoospores, when placed in fresh water. Frequently, however, a certain number fail to form zoospores but germinate directly by a hypha. In sporangia, from their natural habitat on the leaf, the germ-tube arises from the papilla, giving a stout branching hypha, which may form a mycelium or give secondary sporangia after a short growth. This type of germination will be more fully described (from cultures) below.

Some observations were made on the formation and discharge of the zoospores. The ripe sporangium contains a single vacuole of variable size. This is at first irregular and changes shape with the slow movements of the intersporangial protoplasm; then it becomes spherical and ultimately disappears suddenly. The protoplasm itself is at first coarsely granular and with a tendency to form clumps. Later on it becomes more finely granular and after the discharge of the vacuole it is almost homogeneous. About five minutes after the vacuole disappears, the first cleavage lines of the spore-origins become visible and the protoplasm contracts slightly so as to leave a clear space just inside the wall. Soon after, discharge occurs, in the manner so often described for *Phytoph-*

[1] Butler, E. J. *loc. cit.*, p. 121 and, Barrett, J. T. Development and Sexuality of some species of *Olpidiopsis*, Ann. of Bot., XXVI, 1912, p. 215.

thora, the spores being fully demarcated and provided with cilia before they emerge to the outside (Pl. IV, Fig. 3). Often two or more zoospores remain attached, in extreme cases forming a mass in which the individual spores cannot be distinguished. At least ten spores must have been included in some of the masses expelled from feeble or contaminated cultures. These masses are provided with several or numerous cilia but always appear to break down and disintegrate without secreting a wall.

The influence of temperature on zoospore formation is marked. A portion of a culture was divided into two parts, one of which was placed in tap water in the cool incubator at 25°C., the other kept in the laboratory at 32°C., both being equally illuminated. Examined after an hour, the former was found to be discharging freely, the latter not at all. The higher temperature, however, merely inhibits zoospore production, without affecting the power to produce them when suitably cooled, as part of a culture which had remained in water for two hours at 30°C., without any but a very few sporangia having discharged, gave very numerous zoospores when placed in the cool incubator.

Light does not appear to influence the formation of sporangia when temperature conditions are suitable. A culture kept in the dark at 25°C. for a month from the date of sowing was found as fully provided with sporangia as any of the cultures in the laboratory. This differs from the results got by Dastur[1] with *Ph. parasitica*, where cultures kept in the dark remained sterile. No observations were made on the effect of light on the emission of zoospores but Coleman and Dastur have found that a certain amount of light is necessary for this process in *Ph. omnivora* var. *Arecæ* and *Ph. parasitica* respectively.

III. Cultures of Phytophthora Colocasiæ[2].

In recent years several species of *Phytophthora* have been successfully cultivated on artificial media. In the present

1 Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant. Mem. Dept. of Agric. in India, Bot. Ser. V, No. 4, 1913.

2 We are indebted to Mr. J. F. Dastur, First Assistant in the Mycological Section, Pusa, for much assistance in obtaining cultures of the fungus.

species considerable difficulty was experienced, but successful cultures were eventually obtained in the following manner. Healthy living corms were placed in contact with fructifying spots on the leaves, the surface of the corm being sliced off at the point of contact. By keeping the surrounding atmosphere saturated, aerial hyphæ developed on the leaf and penetrated the corm. The latter was then removed to a moist chamber and a good aerial growth of the fungus, bearing sporangia, developed after a few days. A healthy living corm was next sterilised by washing well in spirit and flaming off the excess of alcohol. It was then divided with a sterile knife and the cut surface placed so as to touch the aerial hyphæ of the previously infected corm. After 24 hours the sterilised corm was removed to a sterile moist chamber and gave in subsequent days a copious woolly growth. From this pure cultures were obtained by inoculating tubes of French-bean agar. Other tubes of this medium, as well as of corn meal, were inoculated directly from the first infected corms and purified from the bacteria which contaminated the first tubes by successive subculturing. Ultimately pure cultures were grown on the following media :—French-bean agar, corn meal, oat-juice agar, prune-juice agar, and glucose-peptone agar[1]. Cultures on sterilised slabs of Colocasia corm (sterilised by intermittent steaming in the Koch and placed respectively in potato tubes with distilled water, and on the surface of

[1] These media were prepared as follows :—

French-bean agar.—50 grammes dried beans are pounded in a mortar, made up with about 300 c.c. distilled water, boiled for half an hour and strained through fine wire gauze. To this is added 10 grammes agar dissolved in a small quantity of water and the whole is made up to 500 c.c. with distilled water. After heating to mix thoroughly it is strained through fine cloth into tubes and autoclaved. The reaction was + 2 of Fuller's scale.

Corn meal.—Maize meal moistened with distilled water.

Oat-juice agar.—50 grammes crushed oats are cooked with about 300 c.c. distilled water for an hour in the Koch and strained through wire gauze. To this is added 10 grammes agar dissolved in water enough to bring the whole to 500 c.c. After heating to mix it is strained into test-tubes and autoclaved. The reaction was + 2 of Fuller's scale.

Prune-juice agar.—5 prunes boiled in 100 c.c. distilled water for 5 minutes and filtered. To this is added 7·5 grammes agar dissolved in a small quantity of water and, after thorough mixing, the whole made up to 500 c.c. with distilled water, run into tubes and autoclaved.

Glucose-peptone agar.—Glucose 10 grammes, meat extract 2 grammes, peptone 5 grammes, sodium chloride 2·5 grammes, agar 7·5 grammes, water 500 c.c.

moist corn meal and glucose-peptone agar media) failed to grow.

The best cultures were obtained on French-bean agar, corn meal and oat-juice agar[1]. On corn meal the growth was dense and woolly and extended over the whole surface of the medium fairly rapidly. On French-bean agar the mycelium spread less rapidly and was not as dense as the last. On oat-juice agar there was less aerial growth than the others. Prune-juice agar and glucose-peptone agar gave poor thin growths. It is somewhat surprising that steamed Colocasia corm should, in spite of repeated inoculations, have failed to show any growth. In all the successful cultures sporangia were developed, provided the temperature was not too high. Oospores were much less common and were only formed in French-bean agar, buried in the medium, generally several near together at about one-eighth of an inch below the surface.

The sexual stage of several *Phytophthoras* is rarely developed. In the well-known case of potato blight, *Ph. infestans*, it has only recently been discovered (in the United States), in spite of continual searches during the past thirty years. It has never been encountered under natural conditions (the bodies described by Worthington Smith many years ago have not been accepted by mycologists generally as belonging to this fungus) but has been produced in artificial cultures only[2]. The same is the case with the present fungus, as neither in Java, nor in Formosa, nor in India have oospores been found in or on the diseased plants. It is probable that in these two cases the absence of a resting-spore condition is to be correlated with the presence of a mycelium in the tubers, capable of passing over from one season to another.

The characters of the fungus in culture may now be described, little difference, except in the vigour of growth and the presence or

[1] *cf.* Clinton, G. P. Artificial cultures of *Phytophthora*. Conn. Exper. Sta. Report, 1907-08, p. 898, and Oospores of Potato blight, *ib.*, Report 1909-10, p. 760.

[2] Clinton, G. P. Oospores of Potato blight. Conn. Exper. Sta. Report, 1909-10, p. 753.

absence of oospores, being noticed on the three more satisfactory media, mentioned above.

The mycelium covers the surface of the medium rather slowly and forms a white aerial growth, on which are found mature sporangia in about a week or ten days at temperatures of 15 to 20°C. The hyphæ of the aerial mycelium are long, sparingly branched, about 4 to 7μ in breadth and not varying abruptly in diameter (Pl. III, Fig. 4). Septa are frequent, but instead of being formed, as in the higher fungi, regularly from behind forward at a short distance from the growing apex of the hypha, they are laid down irregularly throughout its length, dividing it into compartments which may contain living protoplasm or be empty of all contents. Sometimes a new branch may arise from below a septum, as shown in Pl. III, Fig. 4*a*. The septation increases as the culture ages and in old or badly nourished cultures is very free. In addition to complete partitions, isolated nodules, spines and plates of cellulose are laid down very commonly on the inside of the hyphal wall. Some of these may be seen in Pl. III, Fig. 8*a*. It naturally suggests itself that such cases as these represent early or arrested stages in the formation of complete septa, the process being then of the type known as *Spirogyra* or *Allomyces*. In several cases, however, the septum appears first as a thin plate, such as that shown in *c* of the same figure, and it has not been possible to decide whether this is preceded by a stage of the type figured in *a*. The mature septum usually becomes much thickened, the most common form showing a conical thickening, which in septa dividing full from empty portions of a hypha is always directed to the full side (Fig. 8*b*). Sometimes there is a corresponding depression on the opposite side and in the earlier stages a pore has been distinctly seen in the centre of the septum. The conical thickening marks the subsequent closure of this pore. Septa often develop at the origin of a lateral branch (Fig. 8*d*). All the septa, as well as the hyphal walls and the sporangia, give typical cellulose reactions.

The mycelium which creeps on the surface of the medium, where a densely felted layer is often formed, is more freely branched

than the aerial hyphæ (Pl. III, Fig. 1). The hyphæ which penetrate the medium are still more branched and are much less regular in diameter than the superficial mycelium (Pl. III, Fig. 2). Their ultimate ramifications are much branched, irregularly swollen, for the most part densely filled with protoplasm and sparingly septate (Pl. III, Fig. 3).

The sporangia are borne both laterally and terminally on the aerial hyphæ (Pl. III, Fig. 4). As in those found naturally on the leaf, their stalks are as a rule much narrowed and they break off, with a portion of the stalk attached, very readily. Sometimes, however, especially in secondary sporangia formed by the germination of primary sporangia, the stalk differs little from an ordinary hypha (Fig. 7*a*). Occasionally the insertion is by a broad base or the sporangium may develop as a unilateral swelling in the course of a hypha (Fig. 7*b*, Fig. 6, upper sporangium). Fig. 5 shows a group of sporangia from a French-bean agar culture 20 days old. They are still less uniform in size and shape than those found on the leaf, measuring from 20 to 61 by 15 to 31μ in diameter and varying from almost cylindrical to broadly oval or club-shaped. They are, however, more elongated on the whole than in any other species of *Phytophthora* hitherto recorded. The papilla is always short and blunt and the base is marked at the point of insertion on the stalk by a strong cellulose plug projecting into the sporangium.

Many of the sporangia developed in culture, fall off within the tube, and in the absence of sufficient moisture to allow of zoospore production, most of these germinate as conidia by putting out one or more germ-tubes. In older cultures, a considerable proportion of the growth consists of hyphæ which have arisen from the first formed spores. This secondary growth again bears sporangia and it is at least conceivable that under certain conditions there may be a continuous succession of the vegetative and asexually reproductive stages of the fungus in cultivated soils, not only in this species, but in *Phytophthora infestans* and other species whose persistence from year to year, without any form of resting-spore, having been discovered under natural conditions, is still difficult

of explanation. It is no longer widely believed that potato blight begins in the new crop by the extension of mycelium from the tubers, where it has remained in a dormant condition since the previous crop was lifted, into the young shoots, where aerial sporangia are developed. De Bary's[1] early experiments showed, no doubt, that this course is sometimes followed. Recent investigators[2] hold that it is certainly rare and insufficient to explain the normal occurrence of the disease in the field. Clinton further notes that the first spots develop in leaves in contact with the ground and believes that the primary attack comes by direct infection of the leaves from the soil. It is true that *Phytophthoras* do not appear to have been cultivated successfully using sterilised soil as a medium, but sterilisation may injure the soil regarded as a culture medium. The allied genus *Pythium* is quite commonly present in garden soils but as the mycelium is easily overlooked, special methods are required for its isolation, and the same holds good for other genera.[3]

The germ-tubes which arise from sporangia in cultures grow not only from the papilla but, when more than one are present, from any part of the spore wall (Pl. III, Fig. 12). Frequently secondary or even tertiary sporangia are formed after limited growth of the tube (Pl. III, Fig. 13). Sporangia that germinate in this fashion are often traversed by cellulose walls dividing the cavity into two or more compartments.

Chlamydospores are frequently formed in culture. They are spherical, spore-like bodies with a smooth, amber-coloured wall which may be 3μ thick and which takes a clear orange-yellow colour when treated with Schulze's solution. They may be terminal or intercalar or formed by a lateral swelling of the hypha (Pl. III,

[1] De Bary, A. Researches into the Nature of the Potato Fungus (*Phytophthora infestans*). Journ. of Botany, n. s., V, 1876.

[2] Clinton, G. P. Downy mildew of potatoes. Conn. Exper. Sta. Report, 1905, p. 304. Pethybridge, G. H. Considerations and experiments on the supposed infection of the potato crop with the blight fungus (*Phytophthora infestans*) by means of mycelium derived directly from the planted tubers. Proc. Roy. Dublin Soc. XIII, n. s., No. 2, 1911. Jones L. R., Giddings N. J., and Lutman B. F. Investigations of the Potato Fungus (*Phytophthora infestans*). U. S. Dept. of Agri. Bur. of Pl. Indus. Bull. 245, 1912.

[3] Jensen, C. N. Fungus flora of the soil. Cornell Univ. Agric. Exper. Sta. Ithaca, Bull. 315, 1912, p. 420.

Fig. 9). They vary from but little more than the breadth of the hypha, to over 30μ in diameter. They are developed both in the basal part of the superficial mycelium and also embedded in the depth of the medium. They are quite distinctive bodies, not resembling in any way either the sporangia or the sexual spores of the fungus. The only other species of *Phytophthora* in which bodies which can be accurately identified as chlamydospores have been found appear to be *Phytophthora Faberi* Maub., in which Rorer[1] has briefly described their characters and Petch[2] also appears to have seen them, and *Phytophthora parasitica* Dastur[3], in which they closely resemble those of the present species. It is not impossible that the bodies described as parthenogenetic oospores in several species are really chlamydospores. Thus several of the figures of the "oospores" of *Phytophthora Faberi* given by Coleman[4], especially Pl. XVIII, Fig. 8, probably represent chlamydospores. So also it is possible that some of the "parthenogenetic oospores" of *Phytophthora infestans*, described by American authors, belong to the same category. In *Pythium palmivorum* the "resting spores" were at first taken for oospores and only later was their true character made out[5]. This species occupies almost an intermediate position between the genera *Pythium* and *Phytophthora* and its resting spores are evidently homologous with the chlamydospores of the latter.

The shape, the thickness and colour of the wall and its reaction with Schulze's solution, the absence of a papilla and the position on the bearing hypha, serve to distinguish these bodies from sporangia. From sexual spores they are equally distinguishable by the insertion on the hypha, more often intercalar than terminal and

[1] Rorer, J. B. Pod Rot, Canker and Chupon-Wilt of Cacao. Bull. Trinidad Dept. of Agric., IX, No. 65, 1910, pp. 13 and 15.

[2] Petch, T. Cacao and Hevea Canker. Circ. & Agric. Journ. of Roy. Bot. Gard., Ceylon, V, No. 13, 1910, p. 153.

[3] Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant, Mem. Dept. of Agric. in India, Bot. Ser. V., No. 4, 1913.

[4] Coleman, L. C. Diseases of the Areca Palm, I, Koleroga. Bull. Dept. of Agric. Mysore State, Mycol. Ser., No. 2, 1910.

[5] Butler, E. J. An account of the genus *Pythium*. Mem. Dept. of Agric. in India, Bot. Ser. I, No. 5, 1907, p. 82 and Bud-rot of Palms in India *ib.*, III, No. 5, 1910, p. 255.

never on a special lateral stalk, the absence of an oogonial wall distinct from that of the spore, the absence of the antheridium, which is large and persistent in the sexual stage, the frequent occurrence in the superficial mycelium, whereas oospores are only produced buried in the medium and the variation in size in the same culture, which is much greater than with the sexual spores.

Their germination has not been followed. That they do not form zoospores is probable from the absence of a papilla and the fact that empty cases were not seen. They seemed to germinate by putting out a germ-tube in some cases but it was difficult to be certain that these were not intercalar chlamydospores and the supposed germ-tube merely the continuation of the stalk.

Oospores have been produced in considerable numbers in some of the cultures. They have been found hitherto only in French-bean agar, and in cultures which all belonged to the same strain, *i.e.*, arose from the same original parent. They continued to develop in sub-cultures carried on over a period of six months and equally whether grown for several generations on this medium or when grown for one or more generations on other media such as corn meal or prune-juice agar and again transferred to French-bean agar. They were produced at laboratory temperatures during the coldest and hottest parts of the year, in a range of temperature from 15° to 30°C., daily mean.

The oogonia are found only in the entirely submerged mycelium, generally in a layer about one-eighth of an inch from the surface and are at once recognisable by the prominent and characteristically shaped antheridium (Pl. IV), which persists for months. Their development presents points of great interest, which were detected in this species almost simultaneously with those of the oogonia of *Phytophthora parasitica* Dastur, with which they agree in their main features. The antheridium is first formed as a club-shaped swelling at the end or near the end of a hypha, possibly sometimes also by direct lateral swelling in the course of the latter. When mature it is cut off by a distinct septum to form the male cell. To it now grows a branch, usually a lateral outgrowth from

a neighbouring, but distinct, hypha. This is the oogonial origin. As the apex reaches the wall of the antheridium, it indents it and then penetrates into the antheridial cavity. Usually it pursues a straight course to the opposite wall, which it again pierces to emerge on the further side. Sometimes it bends within the antheridium so that the point of exit is not opposite that of entry. The free apex now at once swells up into the oogonial cell, the swelling sometimes extending back to a part of the stalk within the antheridium which is either depressed into a cup-shaped hollow in which the oogonium sits or broadened out where the stalk emerges (Pl. IV, Figs. 4 to 12). The oogonium remains closely applied to the antheridium at its basal part and is apparently fused with it over a considerable extent. When the oogonial cell has reached its full size it is cut off by a septum, which is generally formed in the part of the stalk within the antheridium (Pl. IV, Figs. 6 and 13). The contents now contract away from the oogonial wall to form the oosphere, which ultimately develops a thick wall and lies loose in the oogonium, which it does not completely fill. At what stage fertilisation occurs could not be determined, no fertilisation tube having been detected.

So far as could be determined the oogonium arises always from a different hypha from that which bears the antheridium. Sometimes, however, the stalks of both organs swell up and a tangled mass results which prevents their origin being ascertained (Pl. IV, Fig. 14). In most cases where the oogonial stalk could be followed back it arose as a lateral branch from a larger hypha. In one case (Pl. IV, Fig. 11) the oogonial stalk became swollen and gave off two short branches which pierced two antheridia and bore two oogonia.

After the oogonium forms, the wall of the antheridium thickens somewhat, without becoming coloured, and remains clearly visible for a long time. The oogonial wall also thickens, sometimes considerably, and takes a yellow colour. The oogonial stalk below the antheridium is less persistent and in many cases disappears so as to give the impression that the oogonium arises as an ingrowth

from the wall of the antheridium which, after traversing the cavity, emerges on the opposite side. In some cultures a sort of secondary thickening develops on the outside of the oogonium, the outline of which becomes indistinct and granular or roughened (Pl. IV, Fig. 14). A similar condition has been observed in *Phytophthora parasitica*[1] and *Ph. infestans*[2].

The oogonia are subglobose and measure on an average 29·5μ in diameter (limits 24 to 35μ). The oospores are almost exactly spherical and average 23μ in diameter (limits 20 to 28μ). In these measurements a few abnormally large oogonia, in which oospores were not developed but which had normal antheridia, are omitted. They measured up to 41μ in diameter. So also in one or two tubes some very small spores were found with oogonia only 19μ and oospores 15μ in diameter. These were colourless and were probably degenerate forms. The antheridia vary considerably in size, being from 7 to 13μ in their longest diameter.

Germination of the sexual spore has not been observed.

IV. Inoculation experiments with Phytophthora Colocasiæ.

Raciborski[3] tried to infect healthy leaves of *Solanum tuberosum* and *Nicotiana Tabacum* with sporangia of the *Phytophthora* on *Colocasia*, but without success.

Sawada[4] successfully infected Taro (*Colocasia antiquorum*) and "water-potato" (? *Colocasia antiquorum* var.) with sporangia taken direct from a leaf and germinated in distilled water. He failed to infect similarly "Hasu-imo" (? *Colocasia indica* Hassk.) and "Manshu-imo" (? *Alocasia macrorhiza* Schott.), though some small non-spreading spots developed at the seat of inoculation.

On its proper host *Phytophthora Colocasiæ* is highly infective. Inoculations on the leaves with living active zoospores in distilled

[1] Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant. Mem. Dept. of Agric. in India, Bot. Ser., V, No. 4, 1913.

[2] Clinton, G. P. Oospores of Potato blight. Conn. Exper. Sta. Report, 1909-10, p. 770.

[3] Raciborski, M. Parasitische Algen und Pilze Javas, I, 1900, p. 10.

[4] Sawada, K. Infection of Taro. Rep. from Special Reports of the Formosan Agric. Exper. Sta., II, 1911, p. 7 of the reprint.

water gave in some cases visible signs of infection within six hours. Few parasites are known which produce such rapid results as this. In twenty-four hours brown spots at the point of inoculation were well developed. On the second day the patches were larger and yellow drops of liquid were oozing out on their surface. On the third day sporangia were formed. Controls kept under similar conditions showed no sign of disease. The germ-tubes from sporangia which germinate direct as conidia were also found capable of penetrating the leaf, entering across or between the lower epidermal cells.

Infection from zoospores takes place as shown in Plate II, Figs. 2 and 3, which were taken 24 hours after inoculation. The germ-tube pierces the cuticle, frequently swelling up or running for a short distance in the thickness of the outer covering of the epidermis (Fig. 2). It then either enters the epidermal cell or passes down in the partition wall between two cells. In the mesophyll it pursues a strictly intercellular course, sending haustoria into the neighbouring cells. When fructification is about to commence hyphæ from the intercellular mycelium of the mesophyll again penetrate the epidermal cells to reach the surface (Figs. 1 and 4). They may also, as shown in Fig. 1, emerge through stomata or at the junction between adjoining cells.

Inoculations were next undertaken on a number of plants known to be attacked by previously described species of *Phytophthora*. Excepting *Colocasia* none of the *Araceæ* appear to serve as hosts for the genus, several species of which, however, have a wide range of hosts.

The following table gives the details of these inoculations, all of which were made from pure cultures by sowing some of the sporangium-bearing mycelium in distilled water and using the suspension for the inoculations as soon as the water contained plenty of swimming zoospores. The plants were grown in pots and the inoculations done by placing a drop of the suspension on the unwounded or wounded leaf or stem of the plant and covering with a bell jar. The drops generally persisted for at least 48 hours, which

is ample to allow of infection. Leaf inoculations were always done on both surfaces. The wounding, except where otherwise stated, consisted in scraping away the epidermis with a sterile knife. Controls were kept in every case, those of potato being previously wounded on the leaves, the rest unwounded. These were placed as nearly as possible under the same conditions as the inoculated plants, well watered on the green parts and covered by bell jars. All the inoculations in this table were done at laboratory temperatures, which ranged from about 25° to 30° at the time. At the latter temperature the sporangia were sown in cooled water to secure zoospore emission, which does not take place freely at temperatures approaching 30°C. (see p. 241).

Inoculations with Phytophthora Colocasiæ Rac.

Name of plant.	Previously known to serve as host for :—	Nature of inoculation.	Results.
Solanum tuberosum. A mature plant.	*Ph. infestans, parasitica.*	One shoot inoculated on a leaf and at the apical bud. Unwounded.	Negative.
Ditto ..	....	Several leaves, petioles and the apical bud inoculated. Unwounded. Also one wounded leaf.	Do.
Ditto. A young plant.	....	Several leaves inoculated. Unwounded. Also several wounded leaves and the young stem after removing the epidermis.	The wounded leaves developed a wet rot, which rapidly spread to the petiole, causing their collapse. On microscopic examination hyphæ of *Phytophthora* were observed penetrating the epidermis at the margin of the wound, both through stomata and directly across the cells, and were also seen within the leaf tissues. The wounded stem developed a brown discolouration which did not extend. Unwounded leaves gave negative results.

Name of plant.	Previously known to serve as host for :—	Nature of inoculation.	Results.
Solanum Melongena. 4 young plants in a pot.	*Ph. omnivora* var. *Arecæ, parasitica, Faberi.*	A number of unwounded leaves inoculated, also one at a perforating wound.	Negative.
Lycopersicum esculentum. A mature plant.	*Ph. infestans, omnivora* var. *Arecæ, parasitica, Faberi.*	One shoot inoculated on several leaves and petioles. Unwounded.	Do.
Ditto ..	....	Several leaves inoculated. Unwounded.	Do.
Ditto ..	....	Several leaves and petioles wounded and inoculated.	A brown dry rot developed in the neighbourhood of some of the wounds but this did not spread except in one leaf, which dried up slowly. Infection appeared to be usually limited to the wounded area. The petiole inoculations gave no results.
Syringa vulgaris. 2 small plants in pots.	*Ph. Syringæ, omnivora, parasitica.*	Unwounded leaves and buds inoculated.	Negative.
Nicotiana Tabacum. A mature plant.	*Ph. Nicotianæ* ..	Three young leaves and one older inoculated. Unwounded. Also one leaf at a perforating wound.	Do.
Ditto ..	....	Several unwounded leaves.	Do.
Jasminum pubescens. A rooted cutting from a mature plant.	*Ph. Syringæ* known on *Jasminum nudiflorum.*	As in *Syringa vulgaris.*	Do.
Jasminum Sambac. A rooted cutting from a mature plant.	....	Ditto ..	Do.
Ricinus communis. Seedlings in pots.	*Ph. parasitica* ..	The seedlings sprinkled with the suspension of zoospores.	Do.
Ditto Mature plants.	....	Unwounded leaves and stems inoculated.	Do.

Name of plant.	Previously known to serve as host for :—	Nature of inoculation.	Results.
Opuntia Dillenii. A young plant.	*Ph. cactorum, omnivora* var. *Arecæ, Faberi* and *Syringæ* known on several cacti.	Stems and buds inoculated without wounding.	Negative.
Lepidium sativum. Seedlings in a pot.	*Ph. omnivora.* ..	The seedlings sprinkled with the suspension of zoospores.	Do.
Œnothera biennis. Seedlings in a pot.	*Ph. omnivora, omnivora* var. *Arecæ, Faberi, parasitica.*	As in *Lepidium sativum.*	Do.
Ditto ..	....	Ditto ..	Do.
Clarkia elegans. Seedlings in a pot.	*Ph. omnivora, omnivora* var. *Arecæ, Faberi, parasitica.*	Ditto ..	Do.
Salpiglossus variabilis. Seedlings in a pot.	*Ph. omnivora, omnivora* var. *Arecæ, Faberi, parasitica* known on *Salpiglossus* spp.	Ditto ..	Do.
Schizanthus retusa and mixed spp. Seedlings in a pot.	*Ph. infestans, omnivora, omnivora* var. *Arecæ, Faberi, parasitica* known on *Schizanthus* spp.	Ditto ..	Do.
Fagopyrum esculentum. Seedlings in two pots.	*Ph. omnivora, parasitica* known on *Fagopyrum* spp.	Ditto ..	Do.
Gilia nivalis and mixed spp. Seedlings in a pot.	*Ph. omnivora, parasitica* known on *Gilia* spp.	Ditto ..	Several seedlings drooping after three days. Placed in water on a slide gave a copious growth of *Phytophthora Colocasiæ*, bearing sporangia in 24 hours. Both the cotyledons and the hypocotyl were infected, the latter being as a rule more severely attacked.
Ditto	....	Ditto ..	Several seedlings showed drooping cotyledons after two days. On the third day a number had collapsed. About 60 or 70% of these inoculated died. Hyphæ with sporangia of *Phytophthora Colocasiæ* developed in from 6 to 24 hours, when placed in water.

All the inoculations failed except those on seedlings of *Gilia*, where they succeeded well, and on wounded leaves of potato and tomato. The potato infection was definite, that of tomato less so, as little spreading took place. The controls remained healthy in every case. It is clear that the present fungus is restricted in its choice of hosts and therefore more highly specialised than species such as *omnivora* and its allies.

A few tests were made to determine whether temperature had any effect on the infection of seedlings. Seedlings of *Gilia*, *Clarkia*, *Œnothera* and *Fagopyrum* were used. Small pots containing the seedlings were placed in the cool incubator at 25°C., others being kept in the laboratory, the temperature of which at the time was just over 30°C. Both sets were inoculated with swimming zoospores, which at the former temperature are formed freely but at the latter only if sown in chilled water. The *Gilia* seedlings were attacked both in the cold and warm pots. *Œnothera* and *Clarkia* remained healthy in both. *Fagopyrum* was attacked at the cotyledon of one seedling only, in the cool incubator, but not at all in the warm pot. Temperature variations within the limits which markedly affect zoospore formation, have therefore little influence on the resistance of seedlings of the species tried, to *Phytophthora Colocasiæ*.

V. Systematic position of Phytophthora Colocasiæ.

Raciborski (*l.c.*p. 10) noted that this species appeared to be most closely allied to *Ph. Phaseoli* Thaxt. which it resembles in the size of the sporangia while differing in the characters of the stalk. With *Ph. omnivora* de Bary it agrees in the small number of sporangia formed on each sporangiophore. It is quite different from *Ph. Nicotianæ* Breda de Haan.

Sawada (*l.c.* p. 1) transferred it to the genus *Kawakamia* as *K. Colocasiæ* (Rac.) Sawada. *Kawakamia* is defined by Miyabe[1] as parasitic, without haustoria, with conidiophores emerging from the

[1] Miyabe, Kingo, in Kawakami, T. "Kawakamia, a new genus belonging to the *Peronosporaceæ*," Tokyo, Shôkwabo & Co., 1904.

stomata, never branching from the base of the conidium and terminating with a short slender pedicel-cell; the conidia lemon-shaped with a prominent obtuse beak and falling with the thickened septum between the conidium and the pedicel-cell which forms a peculiar short tail to the spore. The other characters are as in *Phytophthora*, from which genus, according to Miyabe's description, it differs only in the pedicel-cell, a tail-like appendage to its conidium, and in the shape of the conidia and conidiophores. These differences appear to be of very slight systematic value and whatever may be said for Miyabe's type, *Kawakamia Cyperi*, the fungus now under consideration can scarcely be removed from *Phytophthora*. In mycelial characters, haustoria, sporangiophores and sporangia there are no points which cannot be paralleled amongst other *Phytophthoras*, excepting that the pedicel-cell may be of unusual length. It is, however, often very short in culture and such short stalks, remaining attached to the sporangium, are sometimes found in *Ph. infestans*[1] and *Ph. Phaseoli*[2]. The characters of the sexual reproduction, unknown to Sawada, are equally in close similarity to other *Phytophthoras*, such as *Ph. parasitica*. Hence it is not possible to maintain Sawada's nomenclature and the species should be restored to the position given it by Raciborski.

Amongst other *Phytophthoras*, only a few show characters similar to those of *Ph. Colocasiæ*. Of these the chief is *Ph. Phaseoli* Thaxt., a species parasitic on *Phaseolus lunatus* in the United States and Russia. From an examination of Clinton's[3] figures it can hardly be doubted that the peculiar type of development of the sexual organs described above in *Ph. Colocasiæ*, and also by Dastur[4] in *Ph. parasitica*, is also found in *Ph. Phaseoli*. Clinton, indeed, states that for a long time it was difficult to decide whether or not the oogonial thread did not actually penetrate the antheridium

[1] De Bary, A. Die gegenwärtig herrschende Kartoffelkrankheit, Leipzig, 1861, figs. 4, 5 and 9.

[2] Clinton, G. P. Downy Mildew of Lima Beans. Conn. Exper. Sta. Report, 1905, p. 288.

[3] Clinton, G. P. Artificial cultures of *Phytophthora*. Conn. Exper. Sta. Report, 1907-08, Plates LXXIV and LXXV.

[4] Dastur, J. F. On *Phytophthora parasitica* nov. spec., a new disease of the Castor oil plant. Mem. Dept. of Agric. in India, Bot. Ser., V, No. 4, 1913.

and that he was not certain that this does not sometimes occur. The sexual organs themselves approach those of *Ph. Colocasiæ* closely in measurements. So also the absence of branching in the conidiophores is a point of resemblance. But the characters of the external growth on the host differ considerably, that of *Ph. Phaseoli* being conspicuous and forming a white felt on the pods while the *Colocasia* fungus is with difficulty visible to the naked eye. The conidiophores of the former are also many times longer than those of the latter and they produce several sporangia successively, the stalk continuing its growth by a new branch just below the spore, whereas on *Colocasia* usually only one spore is borne on each stalk. The sporangia are shorter in *Ph. Phaseoli* and the basal stalk left attached to the spore, when it is shed, is less distinct. These differences are enough to separate the two species.

From the other species of *Phytophthora* with sexual spores resembling ours, *Ph. parasitica* Dastur is at once separated by its broader sporangia, longer conidiophores and smaller oospores. Inoculation experiments also show that its range of hosts is very much wider. In the group of forms formerly included as *Ph. omnivora* de Bary, two require consideration as having possibly a similar type of development of the sexual organs to our species. These are *Ph. Fagi* as described by Himmelbaur[1] and *Ph. omnivora* var. *Arecæ* Coleman. *Ph. Fagi* as first described by Hartig[2] was figured with the antheridium sometimes arising on a distinct hypha and attached to the oogonium at some distance from its base, sometimes on the same stalk and closely applied to the base, so that the oogonium appears to arise directly from the antheridium itself (Hartig *l.c.* p. 49 and Taf. III, fig. 24 *b.*). Himmelbaur states that the antheridium is attached to the underside of the oogonium, near its base. It appears probable that a penetration of the antheridium by the oogonial stalk sometimes occurs in

[1] Himmelbaur, W. Zur Kenntnis der Phytophthoreen. Jahrb. d. Hamburg Wissensch. Anstalten, XXVIII, 1910.

[2] Hartig, R. Der Buchenkeimlingspilz, *Phytophthora Fagi*. Unters a. d. forstbotan. Inst. z. München, I, 1880.

this species. It is, however, readily distinguished from *Ph. Colocasiæ* by its regularly shaped and smaller sporangia. In the species on *Areca*, as described by Coleman[1], the antheridium arises, in some cases at least, before the oogonium and the latter comes to lie immediately above the antheridium, which is applied at its base. His figures suggest an actual penetration of the antheridium by the stalk of the oogonium (see especially Pl. XVII, Fig. 9 and Pl. XVIII, Figs. 1 and 3). But the oospores are larger than in *Ph. Colocasiæ* and the sporangia broader and borne on more branched conidiophores, which form a dense felt on the nuts. The range of hosts is also considerably wider.

The only other species in which it appears likely that the oogonial origin grows through the antheridium is *Ph. infestans*[2]. Clinton's figures, especially Pl. XXXIX E and Pl. XL, B, E, F and I, are clearly capable of this interpretation. But the oospores are larger than in *Ph. Colocasiæ* and the sporangia smaller and borne on characteristically branched conidiophores and there is no danger of confusing the two species.

In none of the other species of *Phytophthora* which have been described are marked relationships with *Ph. Colocasiæ* evident, either in the sexual or asexual stages. The species is indeed one of the most distinctive in the genus.

VI. Treatment of the disease.

Sawada reports an experiment to test the effect of Bordeaux mixture in checking the disease. Half of an infected field was sprayed with Bordeaux mixture to which soap had been added. A month later the sprayed portion was found less infected than the remainder, although there had been much rain and continued gloomy weather. Before spraying all infected leaves were removed.

Bordeaux mixture has considerable prospects of success, owing to its proved efficacy in allied diseases. The sporangia are

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[1] Coleman, L. C. Diseases of the Areca Palm, I, Koleroga. Bull. Dept. of Agric., Mysore State, Mycol. Ser. No. 2, 1910, and Annales Mycologici, VIII, 1910, p. 591.

[2] Clinton, G. P. Oospores of Potato Blight. Conn. Exper. Sta. Report, 1909-10, p. 753.

produced chiefly on the upper surface of the leaves and are therefore more readily reached by the mixture than if they were confined to the under surface, as often occurs in potato blight. Experiments to test the value of spraying have not been possible, as the disease has not been severe at Pusa since 1909, when its study was commenced. The expense of spraying makes it a method of treatment which could only be practicable in very severe attacks and it is doubtful if it could be brought within the means of the ordinary cultivator in India.

In the early stages of attack, all spotted leaves must be removed and destroyed. This will be effective in delaying the period of severe attack and giving the corms time to form.

Corms kept for seed should be gone through at intervals and any that show signs of rotting, removed. At the time of planting only sound corms should be selected. This is probably the most important point in the control of the disease, as provided that a sufficient rotation is practised, the fungus is likely to disappear from the soil unless reintroduced in infected corms.

Planting in shady localities should be avoided, as there is no doubt that the severity of the attack largely depends on the humidity of the air.

December 1912.

DESCRIPTION OF PLATES I TO IV.

(*Phytophthora Colocasiæ* RAC.)

(Plate I was painted by K. Das ; the microscopical drawings were done in pencil with the aid of the camera lucida by E. J. B., and copied in ink by K. Das.)

PLATE I.

Lower figure : a leaf of *Colocasia antiquorum* Schott. severely attacked by *Phytophthora Colocasiæ* Rac., viewed from above.

Upper figure : a young flower similarly affected.

PLATE II.

Fig. 1. Surface view of a portion of a leaf of *Colocasia antiquorum*, bearing sporangia of *Phytophthora Colocasiæ*. X 320.

Fig. 2. Infection of upper surface of *Colocasia* leaf by germinating zoospores, 24 hours after inoculation. X 930.

Fig. 3. Ditto, showing germ-tubes passing between the epidermal cells, not through them. X 930.

Fig. 4. Emergence of a hypha through an epidermal cell of the under surface of the same leaf as in Fig. 3, third day after inoculation. X 930.

Fig. 5. Part of a section of the corm of *Colocasia antiquorum*, showing hyphæ of *Phytophthora Colocasiæ* both in and between the cells of the parenchyma and also in the vessels. X 200.

PLATE III.

Fig. 1. Branching hyphæ from a bean-agar culture at about the surface of the agar. X 330.

Fig. 2. Ditto from submerged mycelium. X 330.

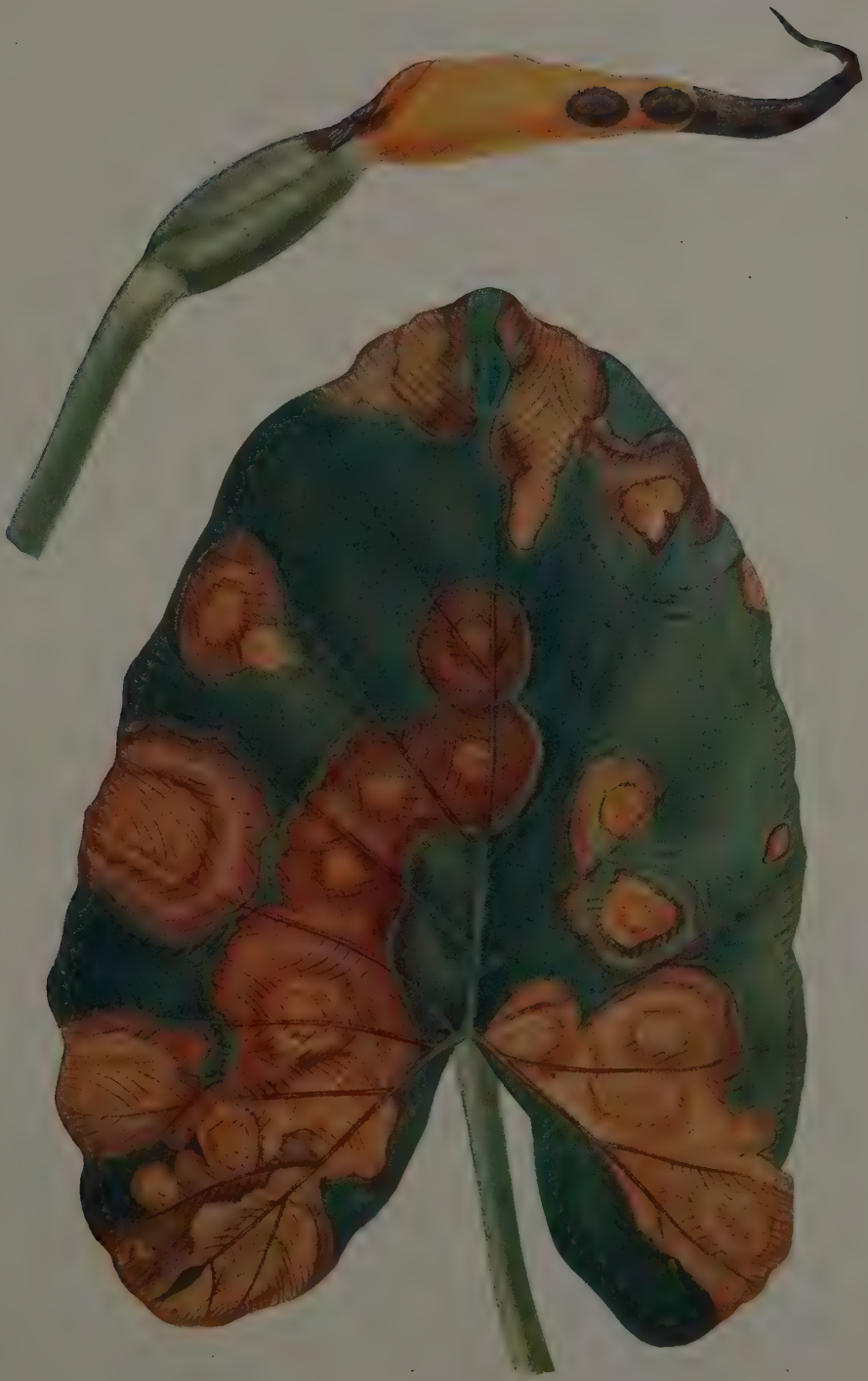
Fig. 3. Ultimate ramifications of submerged mycelium in bean-agar. X 330.

Fig. 4. Aerial mycelium bearing sporangia from a bean-agar culture. The sporangia are both lateral and terminal. At *a*. a branch arises just below a septum. X 330.

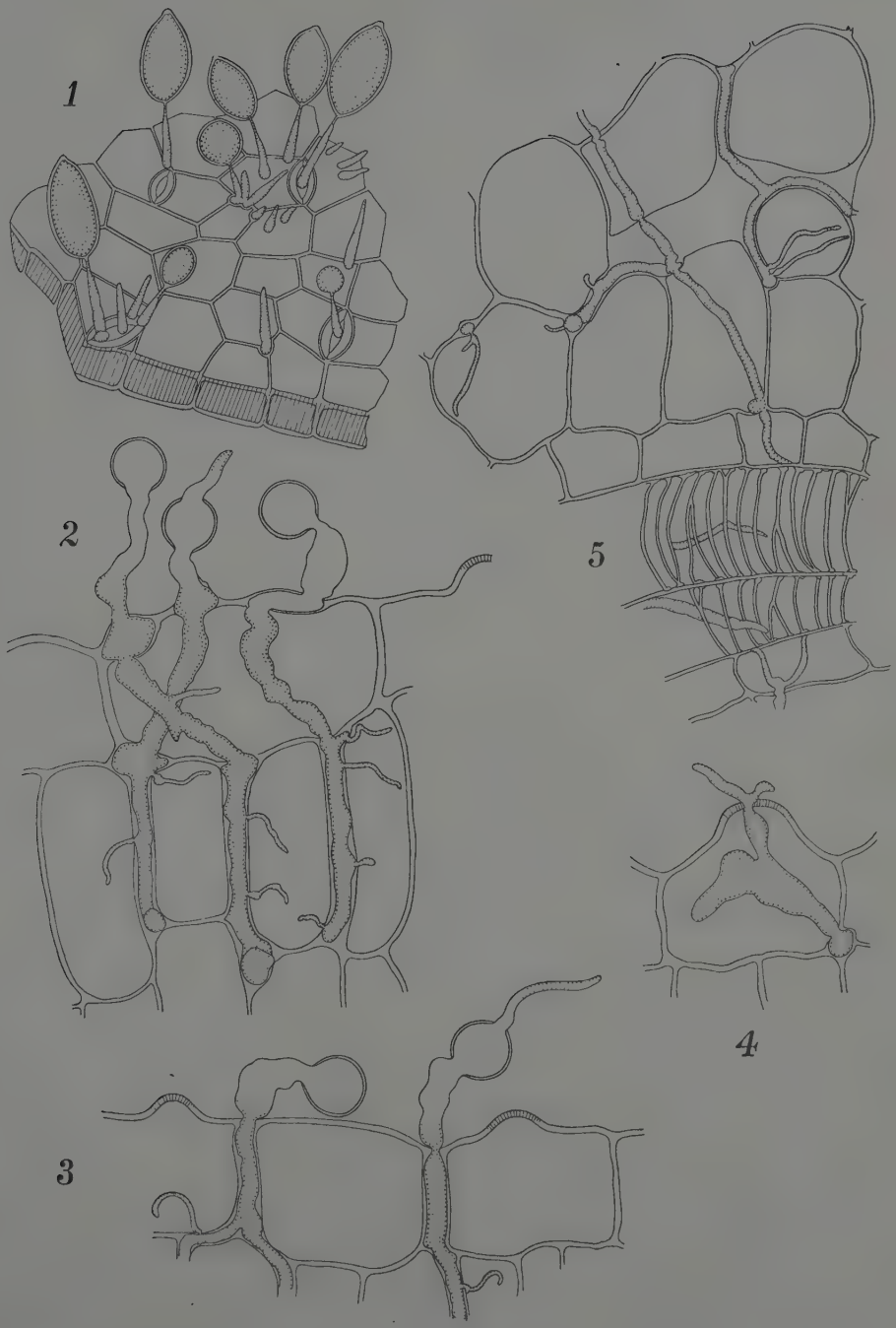
Fig. 5. A group of sporangia from a strong bean-agar culture. X 330.

Fig. 6. Irregularly shaped sporangia, the upper formed without stalk by lateral outgrowth of a hypha. X 330.

Fig. 7. Two sporangia with unusual types of insertion. X 330.

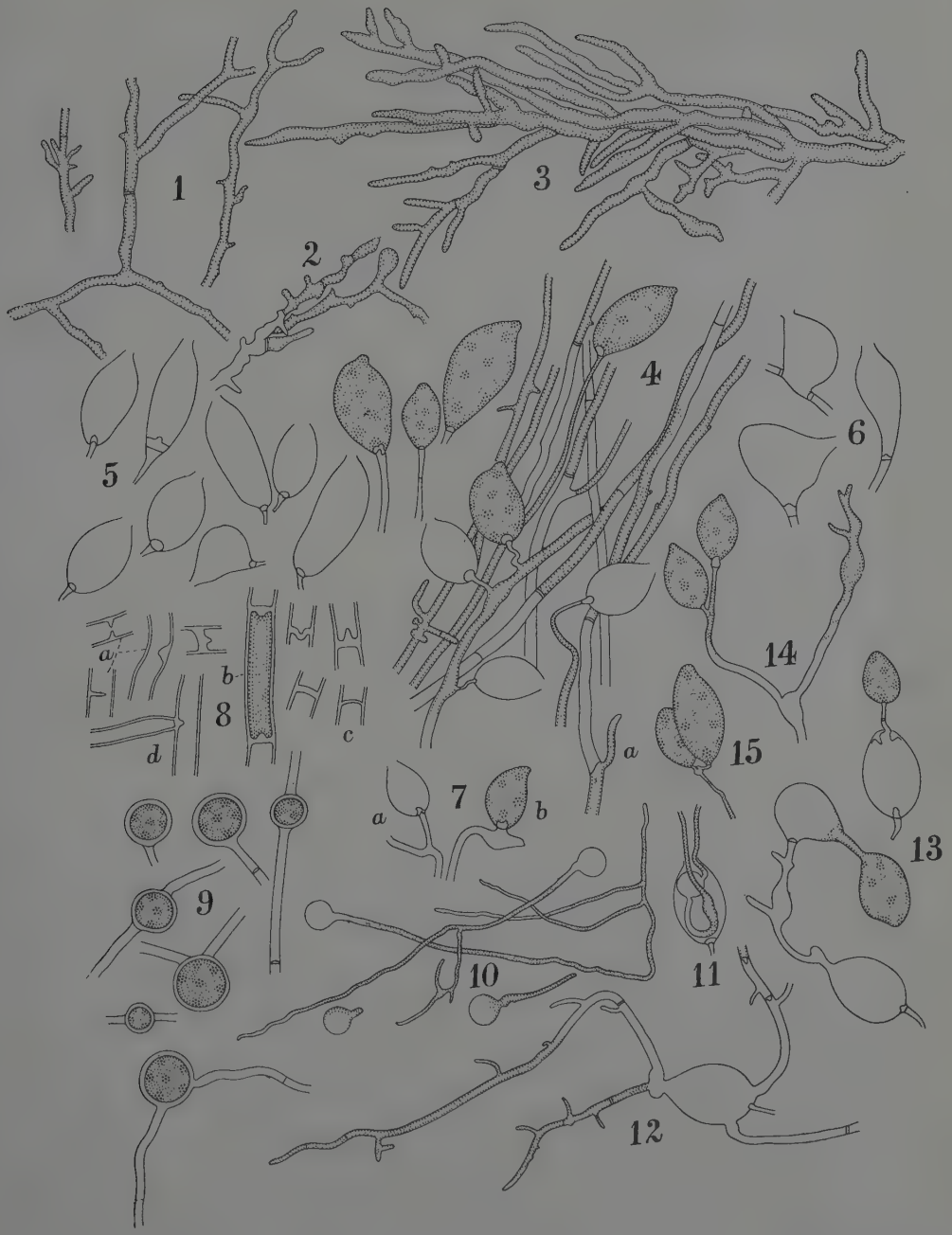


INFLORESCENCE AND LEAF OF COLOCASIA ATTACKED BY PHYTOPHTHORA.



K. D. Das del.

PHYTOPHTHORA COLOCASIAE RAC.



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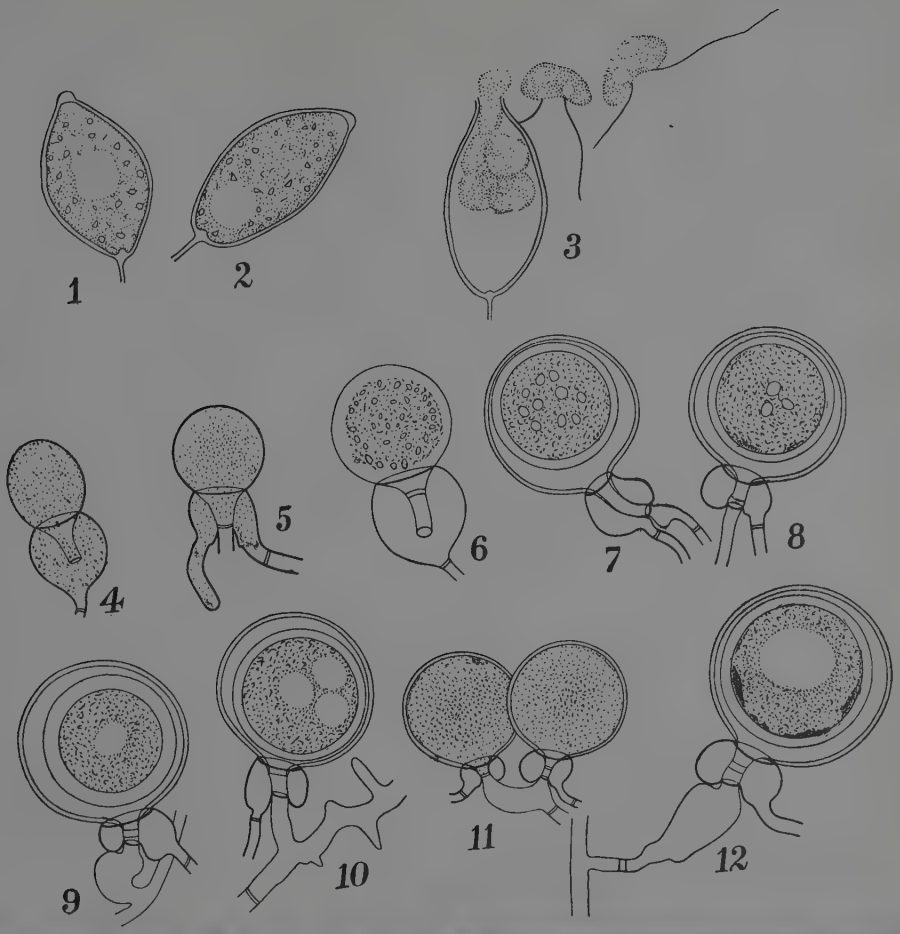
PHYTOPHTHORA COLOCASIAE RAC.



13.

14.

PHYTOPHTHORA COLOCASIAE RAC.

Fig. 8. Details of the septa. *a*. A group showing the formation of cellulose processes on one or both sides of the hypha. *b*. Septa in an old hypha, the space between the septa being filled with cell contents, while above and below it is empty. *c*. A thin regular septum probably of very recent formation. *d*. Septum cutting off a lateral branch. The other septa show various types of thickening. X 930.

Fig. 9. Chlamydospores from oat-juice agar. X 330.

Fig. 10. Germination of the zoospores. X 330.

Fig. 11. Germination of a zoospore while still within the sporangium. X 330.

Fig. 12. Germination of a sporangium with direct production of hyphæ.

Fig. 13. Two sporangia which have germinated with production of secondary sporangia, the lower one having formed a tertiary also. X 330.

Fig. 14. Hypha with sporangia from a corm taken about a month after harvest, cut in two and placed in a moist chamber. X 330.

Fig. 15. Two sporangia arising from the same stalk, the lower formed after the upper, which it has pushed to one side. X 330.

Plate IV.

Fig. 1. A mature sporangium with single irregular vacuole and distinct papilla. X 630.

Fig. 2. A sporangium shortly before discharge, showing the spherical vacuole. X 630.

Fig. 3. Escape of the zoospores. On the right two free swimming zoospores. X 630.

Fig. 4. Formation of the oogonium on a stalk which has penetrated the antheridium. X 930.

Fig. 5. Ditto, later stage. X 930.

Fig. 6. Ditto, later stage, the contents of the oogonium having contracted to form the oosphere. Antheridium empty. X 930.

Figs. 7, 8, 9, 10 and 12. Fully formed oospores with persistent antheridia surrounding the stalks of the oogonia. The origin of the oogonium on a lateral branch from a hypha is seen in Figs. 9, 10 and 12. X 930.

Fig. 11. Two oogonia on a common stalk, the branches from which penetrate two distinct antheridia. X 930.

Fig. 13. Mature oöspore, showing persistent antheridium surrounding the stalk of the oogonium. The antheridial stalk is visible on the right. Micro-photograph.

Fig. 14. Ditto, showing type of oogonium with thickened wall. Micro-photograph.

(We are indebted to Mr. C. M. Hutchinson, Imperial Agricultural Bacteriologist, Pusa, for the micro-photographs reproduced in Figs. 13 and 14, for which a Zeiss 3 mm. apochromatic oil immersion lens was used.)

PYTHIUM DE BARYANUM HESSE.

BY

E. J. BUTLER, M.B., F.L.S.

Imperial Mycologist.

Pythium de Baryanum Hesse, though extremely common as a facultative parasite in garden soils in Europe and the United States, has not, so far as the writer is aware, previously been recorded in Asia. It has recently been isolated from the soil of the Pusa Farm and a short note on its occurrence may be not without interest.

The species was first described by Hesse[1] in 1874, as the cause of a disease, since widely known under the name of "damping off," of seedlings of *Camelina sativa*. It has frequently been observed since on many hosts, and a brief summary of our knowledge of its habits and the plants which it is capable of attacking was published in these Memoirs in 1907[2]. More recently it has come into prominence in Central Europe as a cause of the disease of beet-root (especially sugar beet) known as "wurzelbrand[3]."

In November 1912, some seeds of castor (*Ricinus communis*) which had been sown in pots of unsterilised Pusa soil and had failed to germinate, were examined. A Phycomycete, bearing numerous oospores, was found in the caruncle of the seed. Isolated on boiled ants in the manner described in the writer's memoir on *Pythium*, a copious clean growth was obtained in water. This

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[1] Hesse, R. Ueber *Pythium de Baryanum*, ein endophytischer Schmarotzer, Halle (Inaugr. Dissert.), 1874.

[2] Butler, E. J. An account of the genus *Pythium* and some *Chytridiaceæ*. Mem. Dept. of Agric. in India, Bot. Ser., I, No. 5, 1907.

[3] Peters, L. Ueber die Erreger des Wurzelbrandes, in Busse W. Untersuchungen über die Krankheiten der Rüben, 5, Arb. Kaiserl. Biol. Anstalt für Land-und Forstwirtschaft, VIII, 2, 1911, p. 211. (Gives a full account of the association of *Pythium de Baryanum* with this disease, including references to previous work.)

bore sporangia and conidia on the extra-matrical part of the culture and oospores within the tissues of the ant.

From an examination of these the fungus proved to be the well-known *Pythium de Baryanum*. Subsequent ant cultures gave oospores also on the extra-matrical mycelium. The fungus grew well on boiled *Ricinus* seeds but on this substratum few sporangia were formed, oospores being very numerous within and outside the tissues. Seedlings of garden cress (*Lepidium sativum*), which is well known to be subject to damping off, were attacked in characteristic fashion when grown in soil containing the fungus. The reproductive bodies produced in this substratum were chiefly oospores, which were developed both intra- and extra-matrically. The failure of several observers to obtain the sporangia of this species is possibly to be attributed to the cultures having been made on plant tissues only ; sporangia appear to be rarely produced under such circumstances, whereas on animal tissues they are formed in great abundance.

The mycelium is composed of much branched hyphæ, the main strands being up to 4 or 5μ. in diameter, the lateral ramifications much finer (Pl. V, Fig. 8). Irregular swellings, such as are formed in several other members of the genus, are not common, the hyphæ tapering regularly as a rule. Within the tissues, septa are infrequent, except in the older stages, but the aquatic mycelium is richly septate, especially as soon as the hyphal contents begin to be used up in the formation of the reproductive bodies (Fig. 9).

Sporangia are found in large numbers on the second day in ant cultures. They are terminal or intercalar, the former being mostly spherical, the latter elliptical or irregular (Figs. 8 and 10). The hypha near a sporangium is frequently emptied of its contents, the empty part being sometimes cut off from the full by a septum. Germination in undisturbed cultures generally occurs while the sporangium is still attached to the mycelium. A very prominent beak is formed, generally laterally and about equal to the diameter of the sporangium in length. The sides of this beak are not usually as straight as is figured by Hesse. A single large vacuole is

present in mature sporangia but disappears shortly before zoospore-formation. The latter process is exactly similar to that of other species of the genus, the sporangial contents escaping into a thin-walled vesicle formed at the apex of the beak and maturation of the zoospores taking place within this vesicle (Figs. 11 and 12). Five to eighteen or more zoospores may be given by a sporangium. The zoospores are still figured, by a curious case of persistence of error, in most text-books, as being pear-shaped and uni-ciliate. Even in a recent original study[1], Hesse's old figures of 1874 have been copied. As the fungus is prescribed as a type in the botany syllabus of many institutions and is included in several popular text-books, it is as well to be accurate on this point. When first liberated the zoospore is longer than broad, the two longer sides being unequal in length and the shorter of the two sometimes depressed in the centre, the shape being like a bean somewhat flattened vertically, and with one end more pointed than the other (Fig. 13). From the hollow or hilum two cilia arise and diverge; by their movements the zoospore swims rapidly. As it slows down it becomes shorter and thicker, the two cilia being still distinct. When it comes to rest the cilia are retracted, but their position is still marked for a time by two droplets, perhaps of a fatty nature (Fig. 14). No case was seen of any tendency of the zoospores to divide into two 1-ciliate secondary zoospores, as described by Atkinson[2] in *Pythium intermedium*. It is extremely probable, therefore, that Hesse's figures were based on erroneous observations, the more so that they have not been confirmed by others since. The type of zoospore in *P. de Baryanum* is that normal for the *Peronosporaceæ* as a whole.

On germination the zoospore emits usually a single germ-tube, which grows to a considerable length and branches at the extremity. It is sometimes septate (Fig. 15).

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[1] Peters, L. Eine häufige Stecklingskrankheit der Pelargonien. Gartenflora, 1910, Taf. 1582.

[2] Atkinson, G. F. "Damping off." Cornell University Agric. Exper. Stat. Bull. 94, 1895.

The conidia, which were especially formed in cultures on plant tissues, do not differ in any way from the sporangia, except in their mode of germination. Morphologically they, no doubt, represent sporangia functionally changed in colonising the land. Germination occurs by the protrusion of one or more germ-tubes directly from the conidium (Fig. 16).

The sexual reproduction is well known. Oogonia are formed usually at the ends of lateral branches, but are sometimes also intercalar. A study of the early stages shows that the antheridia are often formed almost simultaneously with the oogonia, by branching of a single hypha (Fig. 5). The oogonial stalk is frequently longer than that of the antheridium, but curved so that the tips of the two hyphæ come into contact (Figs. 4, 5). Sometimes the antheridial cell is cut off while the oogonium is still in free communication with the stalk hypha (Fig. 2). Usually there is only one antheridium, which arises from the same hypha as the oogonium or, less often, from a neighbouring but distinct hypha. Occasionally two antheridia are present and other observers have seen three. Whether all are functional is not known. Sometimes instead of the antheridium appearing on a distinct branch, a part of the stalk just below the oogonium becomes cut off to form an antheridial cell and fertilisation takes place across the basal septum of the oogonium. I have not observed this condition. The cytological details of fertilisation have been fully studied by Miyake.[1]

The following are the measurements of the various reproductive bodies obtained in my cultures, after fixation with osmic acid and mounting in glycerine :—

Sporangia and conidia : 15 to 26μ. (av. 18μ).
Zoospores after coming to rest : 6 to 8μ. (av. 7μ).
Oogonia : on ants 15·5 to 21μ. (av. 18μ.) ; on *Ricinus* and *Lepidium* : 15 to 26μ. (av. 21·2μ).
Oospores : 12 to 20μ. (av. 16μ).

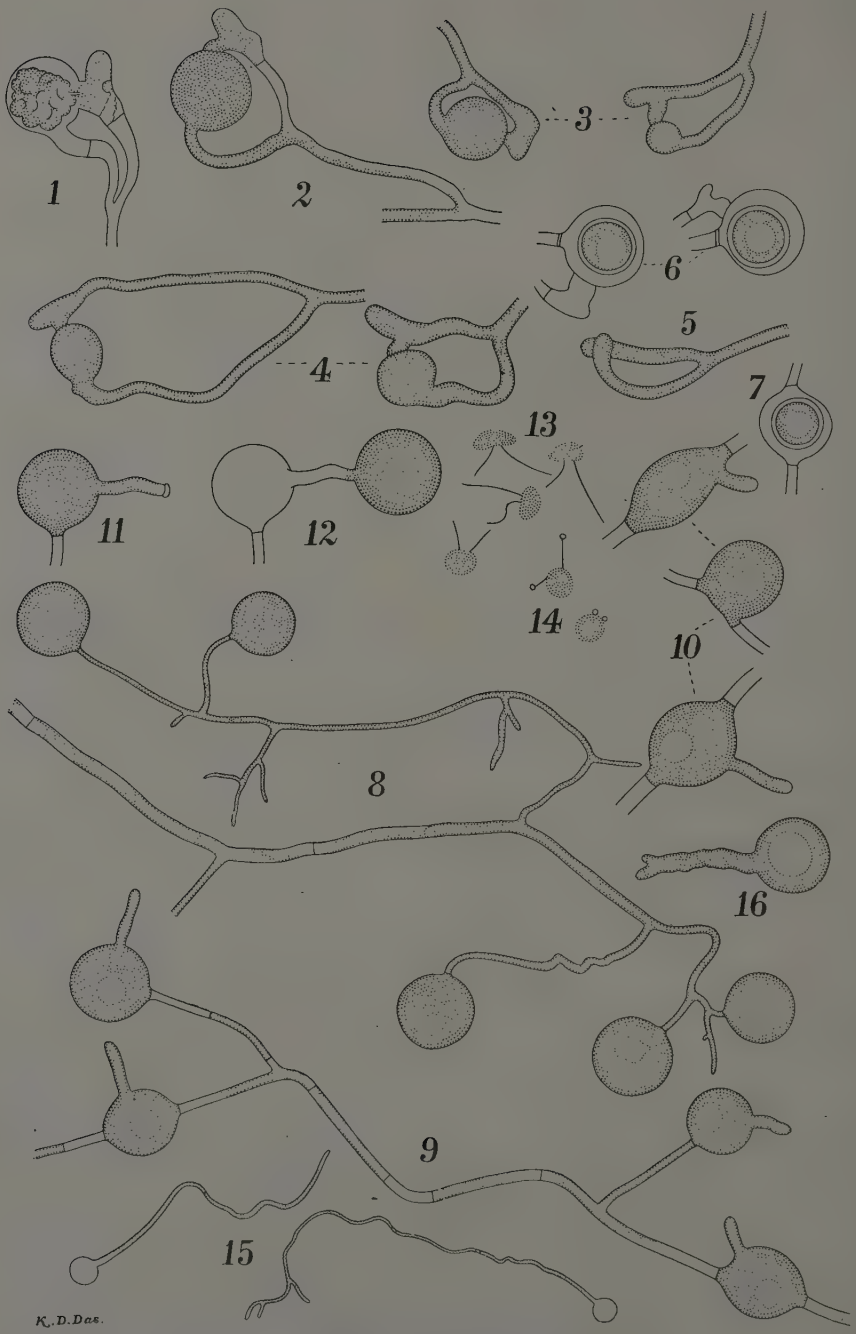
[1] Miyake, K. The Fertilization of *Pythium de Baryanum*. Ann. of Bot. XV, 1901, p. 653.

It is somewhat curious that damping off of seedlings has not been more frequently observed in India. The few cases which had previously come under the writer's notice were due to *Rhizoctonia*. It is possible that this pest of gardens and plant houses in temperate countries is kept in check by climatic conditions in the tropics, since its occurrence at Pusa shows that it is present in soils exposed to as high a temperature as is ordinarily found in the tropics. Its parasitism appears to be as strongly marked here as elsewhere, for not only did it cause germinated cress seedlings to damp off, but it prevented germination in seeds of *Ricinus* in a manner similar to that recorded by Peters[1] in seeds of beet-root.

The treatment of damping off is based on the known conditions which predispose seedlings to this disease. These are overcrowding, growth in damp, stuffy localities, excess of water in the soil, excess of organic matter, especially decaying vegetable debris, and use of the same soil over and over again in plant houses and pots. The addition of sand to the soil, so as to improve its texture, frequent stirring of the top soil, exposure to sunlight and fresh air, evenness of temperature, good drainage and frequent change of soil, will generally suffice to keep seedlings in pots and forcing-beds healthy. In open cultivation the disease has not been found to do much damage, except in the case of beet-root, where several other parasitic fungi aid *Pythium de Baryanum* in causing the condition known as "wurzelbrand."

PUSA:
January 7th, 1913.

[1] Peters, L. Biol. Anstalt, VIII, 2, 1911, p. 225.



K. D. Das.

PYTHIUM de BARYANUM HESSE.

DESCRIPTION OF PLATE V.

(*Pythium de Baryanum* HESSE.)

(*All figures magnified* 640 *diameters.*)

Fig. 1. Oogonium and antheridium during fertilisation.

Fig. 2. Oogonium and antheridium before fertilisation has commenced. The antheridial cell already cut off, the oogonium still in communication with the stalk hypha.

Figs. 3 and 4. Early stages of oogonium and antheridium.

Fig. 5. Very early stage in the formation of the sexual organs. The more curved branch becomes the oogonium, the other the antheridium.

Fig. 6. Two ripe oospores, showing antheridia still attached.

Fig. 7. An intercalar ripe oospore.

Fig. 8. Mycelium with sporangia, before the commencement of germination.

Fig. 9. Older mycelium with sporangia which have commenced to germinate by putting out beaks.

Fig. 10. Intercalar sporangia, showing irregular shapes.

Fig. 11. Sporangium just prior to liberation of zoospores. The vacuole has disappeared and the tip of the beak is furnished with a hyaline cap.

Fig. 12. The same sporangium a few minutes later. The hyaline cap has blown out to a thin-walled bladder in which the protoplasm of the sporangium has accumulated.

Fig. 13. Zoospores during the free swimming period.

Fig. 14. Zoospores after coming to rest and while the two cilia are being retracted.

Fig. 15. Germination of the zoospores.

Fig. 16. A conidium shortly after germination. The germ-tube is beginning to branch at its tip and part of the spore-contents have passed into it.

OBSERVATIONS ON THE DOWNY MILDEW (SCLEROSPORA GRAMINICOLA (SACC.) SCHROET.) OF BAJRI AND JOWAR.

BY

G. S. KULKARNI, L.Ag.,

Mycological Assistant, Bombay Department of Agriculture.

THIS disease is found on three cereals, bajri (*Pennisetum typhoideum*), jowar (*Andropogon Sorghum*), rala (*Setaria italica*), and a fodder grass (*Euchlæna luxurians*). It was first described in India by Dr. Butler[1]. He has dealt with it in detail, giving a full description of the fungus and the changes it induces in the hosts. My observations are chiefly concerned with the field characters of the disease on bajri and jowar, though certain additional interesting facts have been observed in connection with the conidial form on jowar.

BAJRI SCLEROSPORA.

The disease is detected on bajri plants in their very young stage, just after they put forth two or three leaves. The affected plants are pale yellow in colour, and the under surface of the leaves is covered with a white downy material, consisting of sporangiophores and sporangia. These are found on the upper surface of the leaves also to some extent. Ultimately, when the plant is mature, the peculiar malformed head is produced. For the detailed description of the inflorescence and the further effects of the fungus on the host, the Memoir already cited should be consulted. My observations are :—

(1) That infection seems to occur only at or before the seedling stage of the plant, for the disease is confined to plants which show it from the beginning and the secondary

[1] Butler, E. J. Some diseases of Cereals caused by *Sclerospora graminicola*. Mem. Dept. of Agric. in India, Bot. Ser., II, No. 1, 1907.

infection of other plants in the field has not been observed. In order to verify this, 15 inoculations were made on plants not more than 20 days old. In each experiment the moving zoospores were transferred to healthy plants on both surfaces of the leaves and the plant was covered with a bell-jar. But there was no infection in any plant. Besides, in the fields it was observed that the plants in the neighbourhood of the affected one did not take disease, although the leaves of the healthy and the affected were touching each other.

(2) The sporangial stage does not stop after the oospores have commenced to form. It can be found even at the time when the plant is completely mature, along with the oospore state.

(3) Although the malformation of heads occurs only in the plants which are affected from the beginning, it is not uncommon to find plants otherwise apparently healthy producing deformed heads. In other cases a deformed axillary bud is the only sign of disease.

Jowar Sclerospora.

The disease manifests itself on this host in three different ways :

1st form :—Here the attack is just like that on bajri plants. The disease is found on the plant at a very early stage, just after it comes up from the soil. The leaves of the attacked plants are pale yellow in colour, the paleness extending from the base towards the tip, and they are narrow compared to those of healthy plants. Conidiophores and conidia are on both surfaces of the leaves but more abundantly on the lower surface. After five or six weeks, white streaks appear on the top leaves, indicating the beginning of oospore formation, and soon after leaf-shredding appears. The shredding usually begins from the margin towards the midrib from both sides of the leaf and, when complete, only the midrib

remains. The oospores are found even on the sheath of the leaf encircling the stem. The plant never attains the usual height and no head is produced. The disease is confined to the individual plants which are affected from the beginning.

2nd form :—The disease appears after the plant is about two months old. The top leaves of the affected plants become white, the whiteness extending to the base of the side leaves also. Irregular streaks appear on these white leaves, oospores are formed and soon shredding begins. On the lower leaves pale yellow patches appear at the base, on which the conidial stage is found. In this form the conidial stage follows the oogonial or, in some cases, they appear simultaneously. The affected plants rarely produce heads, and the heads, if formed, are much reduced in size and have a few small grains but are not malformed. It is the most common and prominent form in the fields and presumably causes considerable damage. Here also the disease is confined to the attacked plants and does not seem to spread from plant to plant.

3rd form :—The affected plants have on their leaves long, narrow streaks and patches. The streaks are first pale yellow in colour, become orange and finally turn dark brown after the cells of the attacked parts are dead. The conidial stage is found on both the surfaces of the patches, but more on the lower side. The patches appear to spread from the lower leaves to the upper leaves and from the apex of the leaf towards the base. The upper leaves have the patches only towards the tip, the middle ones have more than half of their surface covered with them, while the lower ones have their surface completely covered. The patches are bounded by prominent veins and increase mostly longitudinally. When the attack is severe and complete the whole leaf dries up after turning dark brown. No oospores are formed and no shredding of the leaf is observed. The plants produce normal heads. The disease seems to spread from plant to plant and in the field big patches of affected plants are visible.

From these three different modes of attack it seems that, in bajri and jowar, where the plants show disease from the beginning,

infection occurs at the seedling stage; while in the 2nd form of jowar attack, where it appears all of a sudden when the plant is half-grown, it recalls the mode of attack which occurs in some smuts, where the mycelium is in the grain, grows up in the young plant and appears in the head. In the third case of jowar attack the infection seems to be secondary, as it seems to spread from plant to plant.

Dr. Butler found the sporangial and oogonial forms of the disease on bajri but only the oogonial on jowar. The ripe fruits (oogonial forms) on bajri, and jowar do not differ much in size, shape and colour. According to his measurements the diameter of the oogonium on bajri is 42μ (average of 25 measurements), and that on jowar is 41μ, and on this basis he puts both the forms on jowar and bajri into one species. He says :—" In all systematic works which treat of *Sclerospora*, the characters of the ripe fruit are taken as the basis for classification, and on this basis it is impossible to separate the species " (on jowar) " from *Scl. graminicola*."

Although the oogonial forms do not differ, much difference is found in the conidial forms. There is not a great difference between the conidiophores as to their size, length, etc. In both I found as Dr. Butler described—" broad, rather short stalks, unbranched in the lower part, but usually with a few short thick branches dichotomously or trichotomously formed at the top, and crowned with numerous papillæ of characteristic shape, on which sporangia are borne." But these papillæ of the two show a remarkable difference in their length, the bajri ones being shorter than those of jowar. The maximum length of the bajri papillæ (measurement of 20) was 8·3μ, and that of jowar ones 16·3μ. The sporangia do not differ much in limits of size. The bajri sporangia vary from 19 to 31 by 16 to 21μ in diameter, and the jowar sporangia from 18 to 32 by 16 to 23μ in diameter. But the chief and constant difference is with regard to the shape of the conidia and the way they germinate. The bajri sporangia are broadly elliptical,—slightly pointed at the free end and germinate by liberating zoospores. The jowar sporangia are sub-orbicular, with no papilla at the free end and germinate like conidia

by a germ-tube and not by zoospores. These observations were made for two years, 1910 and 1911, at the kharif and rabi seasons, at different places and on several individuals of each host, and the differences were quite constant.

Further, I made cross-inoculations of both these *Sclerosporas;* bajri plants were inoculated with the conidia of the jowar *Sclerospora* and jowar plants with zoospores of the bajri *Sclerospora.* No infection took place in either case. In order to test this point further, a few affected young bajri plants were transplanted in a jowar plot and a few affected jowar plants in a bajri plot. In both the cases the affected plants grew with disease on them while their neighbours did not take the disease at all.

One reason for the failure of cross-inoculation might of course be that, as noted above, infection probably occurs at the seedling stage and not after. It is also possible that the two forms are each adapted to its own host like " biological forms."

These observations go to show that the bajri and jowar *Sclerosporas* are not one and the same fungus. They differ in their mode of attack and hence in the field characters of the diseases. Although the first form of attack, which occurs probably at the seedling stage, is common to both bajri and jowar, the 2nd and 3rd forms are peculiar to jowar alone. The malformations of the heads found in bajri never occur in jowar and the leaf-shredding which is so marked a character on jowar is much less common on bajri. Each form is not only fixed to its host but has modified morphologically also to a certain degree. These differences seem to me to justify us in regarding the two forms as two distinct varieties, if not species, and the following key is suggested for identification.

Sporangia broadly elliptical, with a papilla at the free end and germinating by zoospores . . . . . *Sclerospora graminicola.*

Sporangia sub-orbicular, with no papilla at the free end and germinating as conidia by a germ-tube . . . . *S. graminicola* var. *Andropogonis Sorghi.*

The way in which the fungus lives from year to year is still a mystery. The conidia lose their vitality after three or four hours.

To test whether the mycelium of the fungus lives in the grain as some of the American mycologists believe, the grains of both jowar and bajri from attacked heads were sown in sterilised soil in pots. The plants did not show any disease and produced sound heads. Such grains were sectioned for mycelium and examined after staining in cotton blue. But no mycelium was seen. The obvious means by which it continues are the oospores. But they have resisted all my attempts to germinate them in the laboratory. As Dr. Butler says: "From the regular manner in which the disease appears in certain places every year, it is highly probable that germination occurs freely in nature under suitable conditions" and it would be possible to germinate them only when those conditions are reached.

Both the *Sclerosporas* occur in all places where the two crops are grown in the Bombay Presidency, with the exception that the jowar *Sclerospora* has not yet been observed in Gujerat.

POONA:
January, 1912.

DESCRIPTION OF PLATES VI AND VII.

(*Sclerospora graminicola* (SACC.) SCHROET).

PLATE VI.

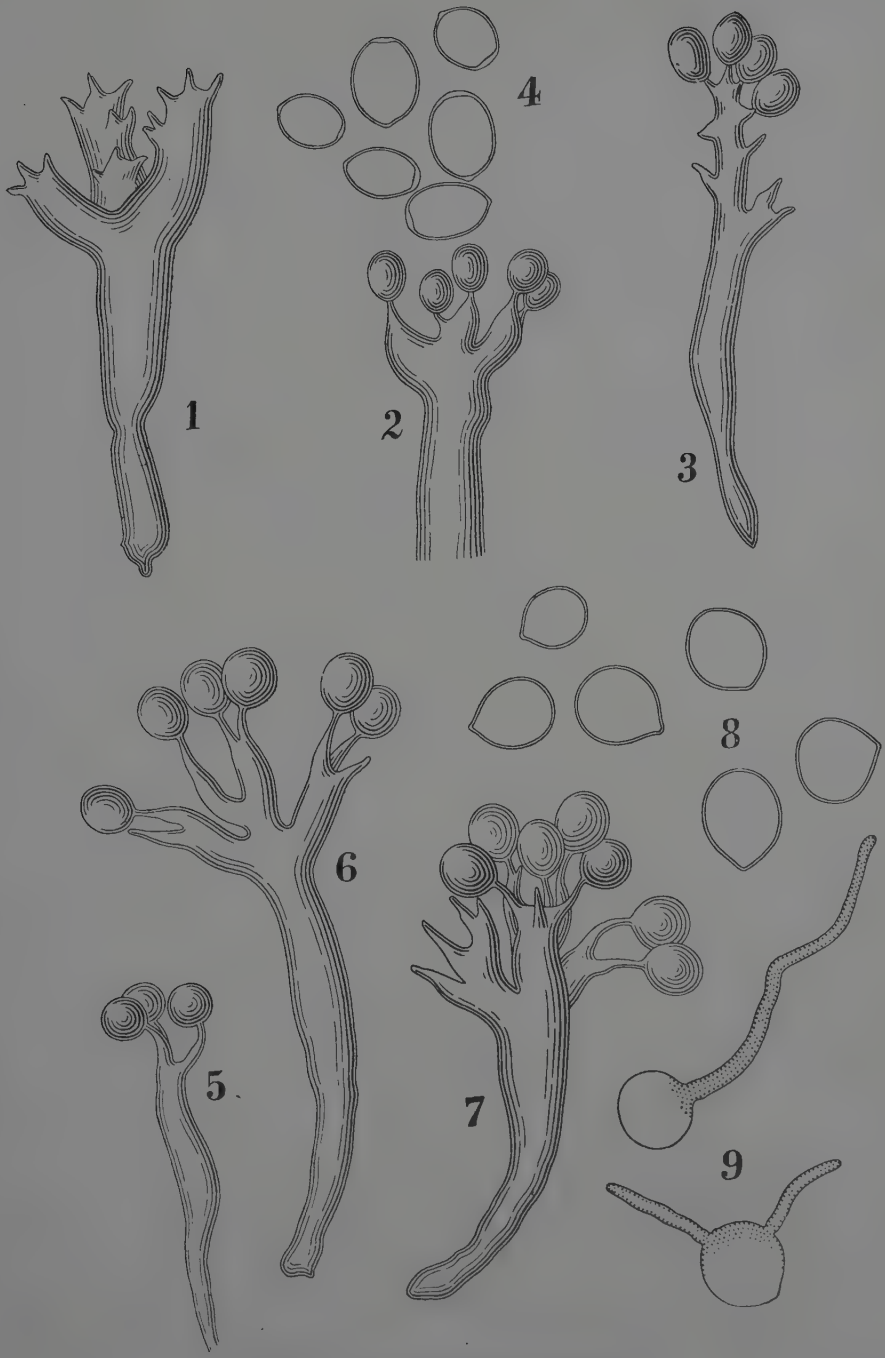
DOWNY MILDEW OF *Andropogon Sorghum*.

Fig. 1. Second form, about the beginning of oospore-formation.
,, 2. First form, early stage, upper surface of leaf.
,, 3. Ditto later stage, lower surface of leaf.
,, 4. Third form, late stage.
,, 5. Ditto very early stage.

PLATE VII.

Fig. 1. Conidiophore of *Sc. graminicola* from *Pennisetum*, showing the short papillæ.
,, 2. Early stage of development of the sporangia from *Pennisetum*.
,, 3. Later stage of ditto ditto.
,, 4. Sporangia from *Pennisetum*. Note the apical thickening which swells to form the papilla of discharge.
,, 5. Early stage of development of the conidia of *Sc. graminicola* var. *Andropogonis Sorghi* from Sorghum.
Figs. 6 & 7. Later stage of ditto. Note the long papillæ on which the spores are borne.
Fig. 8. Conidia from Sorghum.
,, 9. Ditto germinating.

DOWNY MILDEW OF SORGHUM.



SCLEROSPORAS OF PENNISETUM AND SORGHUM.

THE DOWNY MILDEW OF MAIZE

(*Sclerospora Maydis* (Rac.) Butl.)

BY

E. J. BUTLER, M.B., F.L.S.,

Imperial Mycologist.

A disease of maize, not previously known in India, appeared on the Pusa Farm last year (1912). It was found to be identical with one which causes great damage to this crop in Java, where it is termed by the natives "lijer" or "sleepy" disease. Raciborski[1], who first described it in 1897, prophesied its extension from Java to other maize-growing countries in the future; it is certainly of recent appearance at Pusa and it is probable that it has reached India from Java since Raciborski wrote. Up to the present it has not been recorded anywhere else, and we have no information as to its distribution in India or the rest of South-eastern Asia.

The disease becomes apparent before the plants have attained their full growth. The lower leaves are usually normal, but the upper part of the plant is chlorotic, owing to the disappearance of the leaf chlorophyll in long streaks (Pl. VIII). The growth of the plant is checked and the internodes frequently shortened so as to give a bunchy appearance to the head (Pl. IX, Fig. 6). The affected plants are easily recognised at a distance, by their pale colour and stunted bunchy growth. Such plants do not usually produce any grain, though sometimes small cobs are borne. The tassel or male inflorescence is more commonly developed and may be quite normal. The whole plant withers and dies, as a rule, some weeks

[1] Raciborski, M. Lijer, eine gefährliche Maiskrankheit, Ber. d. Deutsch. Bot. Gesellsch. XV, 1897, p. 475.

before the rest of the crop is ready for harvest. Raciborski describes the maize fields in Central Java as having thousands of such diseased plants, the disease being of epidemic intensity. At Pusa, so far, the cases have been few, but the progress of the disease will be watched with some anxiety as the crop is an extremely important one in Bihar and hitherto remarkably free from fungus diseases of any kind.

The cause is a fungus, named *Peronospora Maydis* by Raciborski. It is visible on cursory examination in the form of white downy or woolly patches on both surfaces of the chlorotic streaks on the leaves. These compose the fruiting or sporiferous stage of the fungus, the main body of which occurs as threads or hyphæ within the tissues of the maize plant.

These internal hyphæ are large, unseptate and pursue a tortuous course between the cells of the leaf mesophyll. Lateral swellings indent, and perhaps penetrate, the cell walls, acting probably as haustoria. When fructification is about to begin, hyphæ collect in the sub-stomatal air-spaces and from them small clusters of conidiophores arise through the stomata, coming out on both surfaces of the leaf.

The conidiophores are very thick and rather short, being usually about 20 to 25μ in breadth and 150μ long, the length, however, varying considerably. They are usually unbranched at the base, but fork two or three times near the tip, the end branches being stout and provided each with two or more sterigmata (Pl. IX, Figs. 1 and 3). Each sterigma bears a single conidium, which is spherical when young but broadly oval when mature. The conidia fall off readily when ripe, leaving the conidiophores devoid of spores.

The conidia (Pl. IX, Figs. 1 and 3) are hyaline, thin-walled, not papillate or stalked and measure 28 to 45 by 16 to 22μ in diameter. They germinate readily in water and may be found germinating in large numbers on infected leaves in the field. One or two germ-tubes arise from any part of the spore, giving a sparingly

branched, rather slender hypha, often of considerable length (Pl. IX, Fig. 4). The subsequent fate of the young plant developed from these spores is not known, the species not having been cultivated and no successful inoculations from conidia having been obtained. In hundreds of germinating spores observed, no case of germination by zoospores was seen.

Raciborski described a second spore form, which he stated was found only in the young stem and in the leaf-sheaths of young leaves. This he considered to be an oospore. Similar bodies were found in affected plants at Pusa and increased greatly in numbers as the plants decomposed. Fig. 5 of Pl. IX, shows the character of these bodies, agreeing in every respect with those described and figured by Raciborski. Further examination showed that they were the resting stage of a species of Protozoa allied to *Paramœcium*. This result was not unexpected, as the figure given by Raciborski is quite unlike any spore form known to occur in the *Peronosporaceæ*. A very thorough search was made for true oospores but none were found.

From the conidial stage, which alone is known with certainty, the fungus is a typical *Sclerospora*. It is quite unlike any *Peronospora*. In Berlese's Monograph[1] it is retained in *Peronospora*, as the author had not an opportunity of examining any specimens, but he states that, in his opinion, it is rather a *Sclerospora*. Pammel[2] has suggested that it is identical with *Sclerospora graminicola* (Sacc.) Schroet, the only *Sclerospora* so far known to possess a conidial stage. But a comparison with this species as it occurs in India on *Pennisetum typhoideum*, *Andropogon Sorghum* and species of *Setaria*[3], shows a considerable difference in the size of the conidia, which are much larger on *Zea Mays* than on any of the others.

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[1] Berlese, A. N. Saggio di una monografia delle *Peronosporacee*. Rivista di Patologia Vegetale, X, 1904, p. 219.

[2] Pammel L. H. Bull. Iowa Geological Survey, I, 1901, p. 188.

[3] See Butler, E. J. Some diseases of Cereals caused by *Sclerospora graminicola*. Mem. Dept. of Agric. in India, Bot. Ser., II, No. 1, 1907, and the paper by Mr. G. S. Kulkarni in the present Memoir.

Sclerospora macrospora Sacc. occurs on maize and on many other grasses in Italy[1]. This species is characterised by the absence of a conidial stage and the formation of large oospores within the affected tissues. Diseased plants can only be distinguished from healthy by malformations of the inflorescence, similar to those observed in *Pennisetum* and *Setaria* attacked by *S. graminicola*. There is no alteration in the colour, thickness or consistency of the leaves and no disintegration of the tissues such as causes the peculiar shredding of the leaves noticeable as a result of the action of *S. graminicola*. The symptoms described by Italian observers differ so essentially from those observed in Java and India that it appears to be unlikely that the same fungus can be the cause of both. It is possible, of course, that these differences are due to the occurrence of the fungus in two different stages in Europe and Asia and that, if the conidial stage of *S. macrospora* were to develop in Europe, the affected plants would resemble those observed by Raciborski and myself. Until true oospores are obtained in the eastern form, it will not be possible to decide this point, but in the meantime it appears to be improbable that the cause of "sleepy" disease is *S. macrospora*. It is stated in Duggar's "Fungus diseases of Plants" that *S. macrospora* has been reported on maize in the United States by Fairchild. I have not been able to find the reference and it is not included in Wilson's Host Index of North American *Peronosporales*[2].

With *S. graminicola* there are many points of similarity, especially now that a variety of this species is known which has lost the power of zoospore formation. The mildew of *Andropogon Sorghum*, in the 3rd form of attack described by Mr. Kulkarni in the preceding paper closely resembles that of maize. There

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[1] See Cugini G. & G. B. Traverso. La *Sclerospora macrospora* Sacc. parassita della *Zea Mays* L. Stazione Sperimentali Agrarie Italiane, XXXV, 1902, p. 46, D'Ippolito, G. & G. B. Traverso. La *Sclerospora macrosporu* Sacc. parassita delle infiorescenze virescenti di *Zea Mays* L. *ib.*, XXXVI, 1903, p. 975. D'Ippolito, G. Osservazioni intorno ad alcuni nuovi casi di frondescenza nelle infiorescenze di Granturco, *ib.*, XXXVIII, 1905, p. 998.

[2] Wilson G. W. Studies in North American *Peronosporales*, IV, Host Index. Bull. Torrey Bot. Club, XXXV, 1908, p. 543.

is the same copious production of conidia on chlorotic streaks on the leaves, the same absence of oospores, and the leaves do not split longitudinally, as they do in most cases of infection by *S. graminicola*. But the conidia differ so considerably in size and shape that it is not safe, in the absence of oospores, to consider the two forms to be merely varieties of the one species. For the present, therefore, it appears best to preserve the specific rank of the maize *Sclerospora*, as *S. Maydis* (Rac).

The life-history of the cereal downy mildews, which all belong to the present genus, is the most obscure amongst the *Peronosporaceæ*. Repeated attempts to germinate the oospore have failed. The conidia appear to be short-lived. The symptoms of the disease point to general infection of the plant, similar to what is known in the grain smuts, but whether this infection occurs subsequent to germination or results from mycelium already present in the grain at the time of sowing, is not known. Peglion[1] found the mycelium in the pericarp of wheat grains from deformed ears of plants attacked by *S. macrospora* and was able to trace its growth in plants developed from these seeds, but only when the grain was sown before it ripened fully. I have grown a considerable number of plants of *Pennisetum* from grain gathered from partly deformed ears, but without getting a single case of the disease. Kulkarni reports similar experiments with both *Pennisetum* and *Sorghum*, and neither he nor I have been able to find the mycelium in such grains. Further, I found several typical cases of the green ear disease of *Pennisetum* in a plot sown with grain heated by immersion in hot water to 65° C., for five minutes, a treatment known to be sufficient to kill the mycelium, and even the spores, of the grain smuts. It is probable, therefore, and Peglion himself is of the same opinion, that infection normally takes place after germination. This is most likely to occur through germination of oospores produced in the previous crop.

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[1] Peglion, V. Ueber die Biologie der *Sclerospora*, eines Parasiten der Gramineen. Centralblatt für Bakteriologie, 2nd Ab. XXVIII, 1910, p. 580.

The treatment, on the information at present available, should be directed to preventing the persistence of the parasite in the oospora stage from the previous crop. For this purpose it will be sufficient to remove and destroy all diseased plants before they wither. Such plants can be easily recognised in the field and, so long as the disease remains sporadic, there should be no special difficulty in combating it.

PUSA :

January 15*th*, 1913.

DOWNY MILDEW (SCLEROSPORA MAYDIS (RAC.) BUTL.) ON MAIZE.

PLATE VIII.

DESCRIPTION OF PLATES VIII AND IX.

(*Sclerospora Maydis* (RAC.) BUTL.)

PLATE VIII.

Top of maize plant affected with downy mildew, reduced to two-ninths natural size, and part of leaf of same in early stage, reduced to one-third.

PLATE IX.

Fig. 1. Two conidiophores, branched towards the top, the end branches provided with papillæ, some of which bear spores. X 640.

Fig. 2. Early stages of spore formation. The young conidium is spherical. X 640.

Fig. 3. Much branched conidiophore with conidia which have become separated while mounting. X 320.

Fig. 4. Two germinating conidia. X 320.

Fig. 5. Cystic stage of a Protozoon found in the old leaf-sheaths of maize plants and closely resembling the bodies stated by Raciborski to be the oospores of *Sclerospora Maydis*. X 640.

Fig. 6. A maize plant affected by *Sclerospora Maydis*, showing the manner in which the leaves stand out stiffly from the stalk and the bunched growth at the apex. Note the tip of the tassel appearing between the topmost leaves.